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(72) Inventors; and
(75) Inventors' Applicants (for US only); HINUMA, Shuji [FP/FP];
(75) Inventors' Applicants (for US only); Tsukuba-shi, Ibaraki 305
(FP), HOSOYA, Massaki [FP/FP]; 711-83, Itaya 1-chome,
Tsuchiura-shi, Ibaraki 300 (FP), FUIII, Ryo [FP/FP]; (71) Applicant (for all designated States except US); TAKEDA CHEMICAL INDUSTRIES, LTD. (IP/IP); 1-1, Doshomachi 4-chome, Chuo-ku, Osaka-shi, Osaka 541 (JP).

> 79-303, Kasuga 1-chome, Tsukuba-shi, Ibaraki 305 (IP).
> OHTAKI, Tetsuya [IPIIP]: 79-802, Kasuga 1-chome,
> Tsukuba-shi, Ibaraki 305 (IP). PUKUSUMI. Shoji [IIPIP]:
> 174-302, Namiki 3-chome, Tsukuba-shi, Ibaraki 305 (IP).
> OHGI, Kazuhiro [IPIP]: 161-1206, Umezono 2-chome. Tsukuba-shi, Iberaki 305 (JP).

(74) Agent: MIZUNO, Akinobu; Gloria Miyamasuzaka III 305, 10-7, Shibuya I-chome, Shibuya-ku, Tokyo 150 (JP).

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(54) Tide: G PROTEIN COUPLED RECEPTOR PROTEIN, PRODUCTION, AND USE THEREOF

#### (57) Abstract

DNA primers effective in screening G protein coupled receptor protein-encoding DNA fragments are provided. The primers which are complementary to nucleotide sequences that are in community with (boxologous to) the nucleotide sequences encoding amino acid sequences corresponding to or near the first membrane-spanning domain or the sixth membrane-spanning domain each of known various G protein coupled receptor protein were designed and synthesized. Methods of sumplifying G protein coupled receptor protein-encoding DNAs that is a sumple of the above DNA primers, and novel target G protein coupled receptor protein coupled receptor protein coupled DNAs (Iran-rise, can be efficiently carried out. Human plinitary gland or anygalat-derived and mouse parareas-derived G protein coupled receptor proteins, processes for the coupled receptor proteins proteins, methods of determining ligands for the above G protein coupled receptor proteins, processes for the coupled receptor proteins or proteins or the protein coupled receptor proteins containing that the shove composunds or salts thereof, and antibodies against the above protein coupled receptor proteins or partial peptides thereof are

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#### DESCRIPTION

## G PROTEIN COUPLED RECEPTOR PROTEIN, PRODUCTION, AND USE THEREOF

### FIELD OF THE INVENTION

The present invention relates to novel DNAs which are useful as DNA primers for a polymerase chain reaction (PCR); methods for amplifying DNAs each coding for a G protein coupled receptor protein via PCR techniques using said DNA; screening methods for DNAs each encoding a G protein coupled receptor protein via PCR techniques using said DNA; G protein coupled receptor protein-encoding DNAs obtained by said screening method; G protein coupled receptor proteins which are encoded by the DNA obtained via said screening method, peptide fragments or segments thereof, and modified peptide derivatives thereof; etc.

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The present invention also relates to novel G protein coupled receptor proteins; novel G protein coupled receptor protein-encoding DNAs; processes for producing said G protein coupled receptor protein; use of said receptor protein and said protein-encoding DNA; etc.

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The present invention also relates to novel human amygdaloid nucleus-derived G protein coupled receptor proteins; novel DNAs each coding for said G protein coupled receptor protein; processes for producing said G protein coupled receptor protein; use of said receptor protein and said protein-encoding DNA; etc.

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The present invention also relates to novel mouse pancreatic  $\theta$  cell line MIN6-derived G protein coupled receptor proteins; novel DNAs each coding for said G protein coupled receptor protein; processes for producing said G protein coupled coupled receptor protein; use of said receptor protein and

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said protein-encoding DNA; etc. Further, the present invention relates to novel human-derived G protein coupled receptor proteins (human prinoceptors); novel DNAs each coding for said G protein coupled receptor protein; processes for producing said G protein coupled receptor protein; use of said receptor protein and said protein-encoding DNA; etc.

## BACKGROUND OF THE INVENTION

A variety of hormones, neurotransmitters and the livery control, regulate or adjust the functions of living bodies via specific receptors located in cell membranes. Many of these receptors mediate the transmission of intracellular signals via activation of guanine nucleotide-binding proteins (hereinafter, sometimes referred to as G proteins) with which the receptor is coupled and possess the common (homologous) structure, i.e. seven transmembranes (membrane-spanning regions (domains)). Therefore, such receptors are generically referred to as G protein coupled receptors or seven transmembrane (membrane-spanning) receptors.

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believed that there are many unknown G protein coupled receptor development of pharmaceuticals by investigating the substances neurotransmitters and physiologically active substances, which it has been believed that, if G protein coupled receptor genes action strength, action time, etc., are decided. Accordingly, which act on the receptor. Until now, only several G protein molecules control, regulate or adjust the functions of living or cDNA can be cloned, those will be helpful not only for the physiologically active substances, including specific target cells and organs, specific pharmacological actions, specific bodies. Each molecule has its own receptor protein which is clarification of structure, function, physiological action, specific thereto, whereby the specificities of individual coupled receptor genes or cDNAs have been cloned but it is important role as targets for molecules such as hormones, G protein coupled receptor proteins have a very etc. of the G protein coupled receptor but also for the genes which have not been recognized yet.

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suggested that said protein is a G protein coupled receptor it as a characteristic feature, it is further strongly acid residue alinements are common (homologous) and, by taking protein coupled receptor proteins. In addition, some amino suggested that said protein is within a category of the G When an unknown protein has such a structure, it is strongly are often highly conserved among the receptors. and the amino acid sequences near the membrane-spanning region membrane (membrane-spanning region or transmembrane region) corresponding to the area where the protein passes through the receptor proteins and further that the amino acid sequences a structure is common among all of the known G protein coupled membrane at each region thereof. It has been known that such in the primary structure and pass through (span) the cell seven clusters of hydrophobic amino acid residues are located receptor proteins which have been known up to now is that The characteristic feature of the G protein coupled

Libert, F, et al. (Science, 244:569-571; 1989) reported a method for cloning novel receptor genes by means of a polymerase chain reaction (hereinafter, sometimes referred to as PCR or a PCR technique) for a synthetic DNA primer which was synthesized based upon the information of common amino acid sequences obtained from a comparison among known G protein coupled receptor proteins. Libert, F. et al. used a pair of synthetic DNA primers corresponding to the portions of the third and the sixth membrane-spanning regions. However, in general, the design of primers used for the PCR regulates the molecular species of DNAs which are to be amplified. In addition, when a similarity (homology) in the amino acid

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sequence level is used as a basis, the use of different codons affects on the binding (hybridization) of the primer thereby resulting in a decrease in the amplifying efficiency. Accordingly, although various novel receptor protein DNAs have been obtained using said DNA primers, it is not possible to succeed in amplifying DNAs for all receptor proteins in the prior art.

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Further, the amino acid sequence which is common to from the first to the seventh membrane-spanning regions among 74 G protein coupled receptor proteins was reported by William C. Probst, et al. (DNA and Cell Biology, Vol. 11, No. 1, 1992, pp. 1-20). In this report, however, there is no suggestion for a method in which DNA coding for a novel G protein coupled receptor protein is screened by means of PCR using DNA primers which are complementary to the DNA coding for those amino acid sequences.

It would be desirable to develop DNA primers for PCR techniques which allow selective and efficient screenings of DNAs coding for the areas (regions) more nearer the full length of novel G protein coupled receptor proteins by utilizing the common (homologous) sequence(s) of the G protein coupled receptor protein or the DNA coding therefor.

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It would also be desirable to develop synthetic DNA primers corresponding to the portions of the third and the sixth membrane-spanning regions, said primer being useful in screening for DNA coding for G protein coupled receptor proteins in more selective and efficient manner as compared with a series of the synthetic DNA primers corresponding to the sequences of the third to the sixth membrane-spanning regions as reported by Libert, F. et al.

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G protein coupled receptor proteins are important for investigating substances which control the function of living organisms and proceeding developments thereof as pharmaceuticals. Finding and development of candidate compounds for new pharmaceuticals can be efficiently proceeded by using G protein coupled receptor proteins and by conducting receptor binding experiments and evaluating experiments on agonists/antagonists using intracellular information transmittance systems as indexes. Especially when the presence of a novel G protein coupled receptor protein can be clarified, the presence of a substance having a specific action thereon can be suggested.

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If a novel DNA which codes for a novel G protein coupled receptor protein can be efficiently screened and

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construction of an expression system therefor and the screening isolated, it will now be possible to proceed with the isolation of DNA having an entire coding region, the of an acting ligand.

hypothalamic hormones (hypophysiotropic releasing factors), and organism. Representative examples of the hypothalamic hormones passages for controlling, regulating or adjusting the functions Representative examples of the pituitary hormones include TSH, hormones from the pituitary body (hypophysis) is regulated by the target endocrine glands. A variety of receptor proteins vasopressin, etc. In particular, the secretion of pituitary pituitary hormones released into the blood. Functions which are important for the living body are regulated through this development and growth of a genital system and an individual hypothalamic hormones and peripheral hormones secreted from neurotransmitters with G protein coupled receptors. In the the functions of target cells and organs are controlled by hypothalamo-hypophysial system, the secretion of pituitary system, such as maintenance of homeostasis and control of include TRH, LH-RH, CRF, GRF, somatostatin, galanin, etc. mechanism or a negative feedback mechanism relied on the A hypothalamo-hypophysial system is one of the hormones is regulated according to a positive feedback of organisms relying upon interactions of hormones and present in the pituitary gland play a major role for ACTH, FSH, LH, prolactin, growth hormone, oxytocin, regulating the hypothalamo-hypophysial system.

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It has been widely known that these hormones, factors distributed even in the peripheral tissues to play the role of carrying out the carbohydrate metabolism by secreting not only important functions. The pancreas plays an important role of and receptors are widely distributed in the brain instead of existing only locally in the hypothalamo-hypophysial system. or neuroregulators in the central nervous system. It is "hypothalamic hormones" are working as neurotransmitters This fact suggests that the substances which are called further considered that these substances are similarly

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chiefly by glucose. It has, however, been known that a variety polypeptide, glucagon, amylin, etc.), sugars (mannose, etc.), a digestive fluid but also glucagon and insulin. Insulin is amino acids, and neurotransmitters in addition to glucose. secreted from the eta cells and its secretion is promoted of receptors exist in the eta cells, and the secretion of insulin is controlled by various factors such as peptide hormones (galanin, somatostatin, gastric inhibitory

protein cDNAs. It is not known whether there exist any unknown hormones and neurotransmitters, said receptor proteins playing galanin and amylin, however, there has not yet been reported It has thus been known that in the pituitary gland and in the pancreas are present receptor proteins for many important roles for regulating the functions. As for the any discovery concerning the structure of their receptor receptor proteins or receptor protein subtypes.

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individual substances are defined by the distributions of their variety of actions. To comprehend such complex systems, it is and antagonists capable of regulating the pituitary gland and substances and the specific receptor proteins. It is further necessary to efficiently screen for receptor protein agonists proteins from the standpoint of investigating and developing Accordingly, a substance, in many cases, exhibits an extens% pituitary gland and pancreas, there exist receptor proteins pharmaceuticals, and further to express them in a suitable The pituitary gland and the pancreas are associations of a pancreas, to clarify the structures of genes of receptor For substances regulating the functions of the plurality of functional cells, and the actions of the necessary to clarify the relations between the acting specific to said substance on the surfaces of various target receptor proteins among the functional cells. functional cells of the pituitary gland and pancreas. expression system.

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receptor protein exhibits homology in part of the structure thereof at the amino acid sequence level, an experiment of By utilizing the fact that a G protein coupled

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upon a polymerase chain reaction (hereinafter simply referred looking at DNAs coding for novel receptor proteins relying

to as "PCR") has recently been made. In the central nervous system, many receptor proteins

neurotensin receptor protein, opioid receptor protein, CRF such as dopamine receptor protein, LH-RH receptor protein, receptor protein, CRF receptor protein, somatostatin receptor effects in the central nervous system. clarified that ligands to these receptors exert a variety of are G protein coupled receptor proteins, and it has been protein, galanin receptor protein, TRH receptor protein, etc.

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G protein coupled receptor proteins, and are working as protein, a C5a receptor protein, etc. have been known as such receptor proteins responsive to immunoregulating substances to receptor protein, an MIPIa receptor protein, an IL-8 receptor proliferation and differentiation of nerve cells. factor and a biologically active factor related to the the immune system. IL-6 is both a  $extit{ heta}$  -cell differentiating acts both in the above-mentioned central nervous system and in body. There is, for example, an II-6 receptor protein that play important roles for regulating the functions of the living In the immune system, an  $\alpha$  - or a  $\beta$  -chemokine

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in the central nervous system and in the immune system and are up to the peripheral tissues instead of existing only locally factors and receptor proteins are usually widely distributed producing important functions, respectively. Agonists and as various useful pharmaceuticals. antagonists for these receptor proteins are now being developed It has been widely known that these hormones,

receptor proteins specific to said substance on the surfaces of central nervous system and the immune system, there exist system are associations of a plurality of functional cells, and immune system. The central nervous system and the immune various functional cells of the central nervous system and the distributions of their target receptor proteins among the the actions of the individual substances are defined by the For substances regulating the functions of the

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it is necessary to clarify relations between the acting physiological phenomenon. To comprehend such complex systems, exhibits an extensive variety of actions. Moreover, there functional cells. Accordingly, a substance, in many cases, substances and the specific receptor proteins. is an example wherein many factors play a part in a

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molecules such as hormones, neurotransmitters and cells and organs and has a very important role as a target for physiologically active substances, which molecules control receptor protein is present on the cell surface of living body regulate or adjust the functions of living body cells and As discussed herein above, the G protein coupled

### SUMMARY OF THE INVENTION

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novel DNAs which are useful as DNA primers for a polymerase protein coupled receptor protein using said DNA; screening chain reaction; methods for amplifying a DNA coding for a G methods for the DNA coding for a G protein coupled receptor obtained by said screening method, peptide fragments and G protein coupled receptor proteins encoded by the DNA protein using said DNA; DNAs obtained by said screening method; salts thereof. or segments thereof, modified peptide derivatives thereof or One object of the present invention is to provide

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မ processes for producing said receptor protein; transformants determining a ligand to the receptor protein; screening methods capable of expressing said receptor protein; cell membrane screening method, pharmaceutical compositions comprising binding of the ligand with the receptor protein; kits for said for a compound or a salt thereof capable of inhibiting the fractions obtained from said transformant; methods for an effective amount of the inhibitory compound; antibodies protein or said antibody and use of said receptor protein and against said receptor protein; immunoassays using said receptor encoding DNA. Another object of the present invention is to provide

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against said receptor protein; immunoassays using said receptor screening methods for a compound or a salt thereof capable of protein or said antibody and use of said receptor protein and Yet another object of the present invention is to provide novel G protein coupled receptor proteins which are comprising a DNA coding for said G protein coupled receptor expressed in pituitary glands or pancreatic eta cells; DNAs compositions comprising the inhibitory compound; antibodies transformants capable of expressing said receptor protein; methods for determining a ligand to the receptor protein; cell membrane fractions obtained from said transformant; Protein; processes for producing said receptor protein; protein; kits for said screening method, pharmaceutical inhibiting the binding of the ligand with the receptor encoding DNA.

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Still another object of the present invention is to inhibitory compound; antibodies against said receptor protein; immunoassays using said receptor protein or said antibody and compound or a salt thereof capable of inhibiting the binding screening method, pharmaceutical compositions comprising the coupled receptor proteins; DNAs comprising a DNA coding for obtained from said transformant; methods for determining a producing said receptor protein; transformants capable of expressing said receptor protein; cell membrane fractions Provide novel human amygdaloid nucleus-derived G protein ligand to the receptor protein; screening methods for a of the ligand with the receptor protein; kits for said said G protein coupled receptor protein; processes for use of said receptor protein and encoding DNA.

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protein coupled receptor proteins; DNAs comprising a DNA coding Yet another object of the present invention is to provide novel mouse pancreatic  $oldsymbol{eta}$  cell line MIN6-derived G for said G protein coupled receptor protein; processes for obtained from said transformant; methods for determining a producing said receptor protein; transformants capable of expressing said receptor protein; cell membrane fractions ligand to the receptor protein; screening methods for a

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inhibitory compound; antibodies against said receptor protein; immunoassays using said receptor protein or said antibody and compound or a salt thereof capable of inhibiting the binding screening method, pharmaceutical compositions comprising the of the ligand with the receptor protein; kits for said use of said receptor protein and encoding DNA.

coupled receptor proteins. It is to be particularly noted that The present inventors have succeeded in synthesizing or the sixth membrane-spanning region each of known G protein novel DNA primers based upon the similarity (homology) with 🖞 sequence coding for the first and the sixth membrane-spanning base sequences coding for the first membrane-spanning region there has been no report of a DNA primer pair which has been synthesized paying attention to the similarity with the base region of the known G protein coupled receptor protein,

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succeeded in efficiently amplifying DNAs (DNA fragments) coding (homology) with the base sequences coding for the third or the synthesizing other novel DNA primers based upon the similarity for G protein coupled receptor proteins by means of PCR using coupled receptor proteins. They have also unexpectedly Next the present inventors have succeeded in sixth membrane-spanning region each of known G protein those DNA primers.

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DNA primers based upon the similarity (homology) with the bas region each of known G protein coupled receptor proteins; and upon the similarity (homology) with the base sequences coding for the second or the sixth membrane-spanning region each of They have further succeeded in synthesizing novel amplifying DNAs (DNA fragments) coding for G protein coupled receptor proteins by conducting PCR using those DNA primers. Moreover, the present inventors have succeeded in sequences coding for first or the third membrane-spanning spanning region each of known G protein coupled receptor sequences coding for the second or the seventh membrane-Proteins; upon the similarity (homology) with the base known G protein coupled receptor proteins. They have furthermore and unexpectedly succeeded in efficiently

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prepared via conducting amplifications and analyses of various coupled receptor proteins can be isolated, characterized or Thus, they have found that novel DNA coding for novel G protein fragments) coding for said G protein coupled receptor protein. coupled receptor protein via using amplified DNAs (DNA efficiently cloning full-length DNA coding for said G protein DNA using said DNA primers.

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15 20 selected amino acid sequences which are each common to the primer (SEQ ID NO: 2) which is complementary to the nucleotide coupled receptor proteins and have designed the DNA primer membrane-spanning region of the known individual G protein portion corresponding to or near the first and the sixth primers corresponding to the third and the sixth membranecompared with reported DNA primers (e.g. a set of synthetic DNA sequence coding for the amino acid sequence common (homologous) (homologous) to the first membrane-spanning region and the DNA by Libert, F. et al.) and such instant primers are novel and spanning regions (SEQ ID NO: 60 and SEQ ID NO: 61) as reported Those DNA primers have a different nucleotide sequence as to the area near the sixth membrane-spanning region. (SEQ ID NO: 1) coding for the amino acid sequence common To be more specific, the present inventors have

35 elongation reaction in the PCR, the 3'-terminal region of the nucleotide sequence level (base sequence level) is utilized for instant primers contains the nucleotide sequence which and, from those products, obtained the G protein coupled derived from human brain amygdala, human pituitary gland and nucleotide sequences (base sequences) are matched for as many setting the mixed base (nucleotide) parts wherein their Even in other areas, the similarity (homology) at the is common (homologous) among many receptor proteins. receptor protein cDNAs having the sequence as shown in rat brain, found the amplified products as shown in Figure 17 proteins. Then the present inventors have amplified cDNA nucleotides (bases) as possible among many DNA for the receptor Especially for an object of conducting an efficient

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υī Figure 37, Figure 40, Figure 43 or Figure 46 are novel. Figure 22, Figure 23, Figure 27, Figure 29, Figure 34, coupled receptor protein cDNAs having the sequence as shown in Figure 40, Figure 43 or Figure 46. Among them, the G protein Figure 23, Figure27, Figure 29, Figure 34, Figure 37, Figure 18, Figure 19, Figure 20, Figure 21, Figure 22,

sixth membrane-spanning region each of the known G protein acid sequence common (homologous) to the portion near the sixth NO: 6 and SEQ ID NO: 7) and the DNA primers which are coupled receptor proteins and designed the DNA primers coding amino acid sequences common (homologous) to the third and the complementary to the nucleotide sequence coding for the amino membrane-spanning region (SEQ ID NO: 3; SEQ ID NO: 5, SEQ ID for the amino acid sequence common (homologous) to the third third and the sixth membrane-spanning regions (SEQ ID NO: 60 NO: 9). Again, those DNA primers have different base sequences membrane-spanning region (SEQ ID NO: 4, SEQ ID NO: 8 and SEQ ID or Figure 52. Those cDNAs are novel. coupled receptor protein cDNA having the sequence of Figure 49 pylorus of rabbits using said DNA primer and obtained G protein amplified cDNA derived from the smooth muscles of gastric instant primers are novel and unique. The present inventors and SEQ ID NO: 61) as reported by Libert, F. et al.) and such of synthetic DNA primers corresponding to the sequence of the from those of the DNA primers previously reported (e.g., a set Further, the present inventors have selected the

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second membrane-spanning region (SEQ ID NO: 10) and the DNA protein coupled receptor proteins and designed the DNA primer the seventh membrane-spanning region each of the known G the amino acid sequences common (homologous) to the second and near the seventh membrane-spanning region (SEQ ID NO: 11). the amino acid sequence common (homologous) to the portions primer which is complementary to the base seguence coding for coding for the amino acid sequence common (homologous) to the DNA primers previously reported (e.g., a set of synthetic DNA Those DNA primers have different base sequences from those of Still further, the present inventors have selected

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primers corresponding to the part of the third and the sixth membrane-spanning regions (SEQ ID NO: 60 and SEQ ID NO: 61) as reported by Libert, F. et al) and such instant primers are novel and unique. The present inventors amplified cDNA derived from the smooth muscles of gastric pylorus of rabbits using said DNA primer and obtained G protein coupled receptor protein cDNAs having each the sequence of Figure 55, Figure 56, Figure 72, or Figure 73. Those cDNAs are novel.

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primers have different base sequences from those of DNA primers Furthermore, the present inventors have selected the amino acid sequence common (homologous) to the third membranenear the sixth membrane-spanning region (SEQ ID NO: 15 and SEQ amino acid sequences common (homologous) to the second and the amino acid sequences common (homologous) to the first and the acid sequences common (homologous) to the third and the sixth receptor proteins and designed the DNA primers coding for the spanning region (SEQ ID NO: 10 and SEQ ID NO: 18) and the DNA ID NO: 19). Further, the present inventors have selected the coupled receptor proteins and designed the DNA primer coding for the amino acid seguence common (homologous) to the second amino acid seguence common (homologous) to the parts near the for the amino acid sequence common (homologous) to the first amino acid sequence common (homologous) to the portions near membrane-spanning region each of the known G protein coupled Still further, the present inventors have selected the amino for the amino acid sequence common (homologous) to the parts coupled receptor proteins and designed the DNA primer coding membrane-spanning region (SEQ ID NO: 12) and the DNA primer primers which are complementary to the base sequence coding third membrane-spanning region each of the known G protein which is complementary to the base sequence coding for the membrane-spanning region (SEQ ID NO: 16) and the DNA primer sixth membrane-spanning region each of the known G protein which is complementary to the base sequence coding for the sixth membrane-spanning region (SEQ ID NO: 17). Those DNA previously reported (e.g., a set of synthetic DNA primers the third membrane-spanning region (SEQ ID NO: 13).

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corresponding to the part of the third and the sixth membrane-spanning regions (SEQ ID NO: 60 and SEQ ID NO: 61) as reported by Libert, F. et al) and such instant primers are novel and unique.

Still another object of the present invention is to provide a G protein coupled receptor protein expressed in the pituitary gland and pancreatic  $\beta$  cells, a DNA comprising a DNA coding for said protein, a process for producing said protein, and use of said protein and DNA.

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As a result, the present inventors have succeeded in amplifying expressed by a suitable means permits screening for a ligand to Pancreatic eta -cell strain, MIN 6, with a synthetic DNA primer for efficiently isolating G protein coupled receptor proteinand mouse-derived G protein coupled receptor protein-encoding CDNAs, in determining the partial structure thereof, and have discovered that the above-mentioned receptor protein obtained considered that these cDNA sequences are preserved very well receptor proteins for the same ligand. Based upon the above In order to achieve the above-mentioned aims, the when the G protein coupled receptor protein-encoding cDNA is Open reading frame (ORF) of the receptor protein, hence, to the receptor protein from the living body or from natural or knowledge, the present inventors have discovered that these DNAs make it possible to obtain a cDNA having a full length CDNA derived from the human pituitary gland and the mouse encoding DNA, and have forwarded the analysis. Thus, the present inventors have succeeded in isolating novel human non-natural compounds under guidance of data obtainable in second messengers, etc. and further allows screening for a produce the receptor protein. The inventors have further in the human and in the mouse, and are coding for novel receptor coupling tests or measurements of intracellular compound that inhibits the binding of the ligand and the present inventors have made extensive investigations. receptor protein.

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In one embodiment, the present inventors have carried out PCR amplification of novel human pituitary gland-

that the cDNA has been encoded a novel receptor protein. From analysis of the partial sequence, it has been clarified have subcloned them to obtain a plasmid vector (p19P2). derived cDNA fragments as shown in Figures 22 and 23, and

G translated into an amino acid sequence (SEQ ID NO: 24) the 5' side (first membrane-spanning domain side) and has been among the membrane-spanning domains. The nucleotide sequence corresponding to the first and sixth membrane-spanning regions known G protein coupled receptor proteins in common, i.e., corresponding to seven hydrophobic clusters that exist in the The synthetic DNA primers used for amplifying the cDNA are (SEQ ID NO: 29) has been determined from the primer region at [Figure 22]. As a result, the second and third membrane-

15 20 membrane-spanning domains has been confirmed on the plotting [Figure 58]. Similarly, the nucleotide sequence hydrophobicity plots [Figure 59]. The size of the amplified translated into an amino acid sequence (SEQ ID NO: 25) [Figure 23]. As a result, the presence of the sixth and fifth the 3' side (sixth membrane-spanning domain side) and has been (SEQ ID NO: 30) has been determined from the primer region at

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acid sequence deduced therefrom is represented by SEQ ID NO:

hydrophobicity plotting has been carried out. The results

26 [Figure 34]. Based upon the amino acid sequence,

spanning domains have been confirmed on the hydrophobicity

sixth membrane-spanning domain of the known G protein coupled of bases between the first membrane-spanning domain and the cDNA is about 700 bp which is nearly comparable with the number receptor protein.

has been carried out based upon the amino acid sequence of the are forming one protein family. Therefore, data base retrieval property to some extent at an amino acid sequence level, and the G protein coupled receptor protein family. Moreover, the by S12863) that is shown in Figure 60. This fact tells that included in p19P2). As a result, a high homology has been subject novel receptor protein (protein encoded by cDNA the novel receptor protein of the present invention belongs to receptor protein (rat neuropeptide Y receptor protein encoded exhibited as compared with the known G protein coupled data base has been retrieved using, as a template, the amino G protein coupled receptor proteins exert common

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10 v registered as data to NBRF-PIR/Swiss-PROT and are, usually, are reference numbers that are assigned when they are from which it is learned that a novel receptor protein had protein (P30098) and human-derived NK-2 receptor protein RP-23 (B40470), human-derived ligand unknown K-opioid receptor protein coupled receptor proteins, mouse-derived ligand unknown high homology to the amino acid sequences of the known G been encoded. The aforementioned abbreviations in parentheses acid sequence encoded by the DNA of the invention. It exhibits (JQ1059). However, none of them are in perfect agreement,

15 a full length open reading frame of the receptor protein shows the receptor protein of the present invention has been obtained protein-encoding cDNA fragment (p19P2) of the present receptor protein is represented by SEQ ID NO: 31, and the amino that the nucleotide sequence of a coding region of this sequence analysis of a plasmid (phGR3) carrying the cDNA having from human pituitary gland cDNA libraries. The nucleotide invention, a cDNA having a full-length open reading frame of Next, by using the novel G protein coupled receptor

each called "Accession Number".

30 25 receptor protein. An expression of mRNA for receptor genes cDNA obtained according to the present invention is a has been confirmed that the receptor protein encoded by the has been clarified that the receptor protein of the present are shown in Figure 36. From the hydrophobicity plotting, it human pituitary gland [Figure 35]. been confirmed that the receptor gene has been expressed in the by northern blotting techniques at a mRNA level, and it has encoded by the cDNA of the present invention has been checked seven transmembrane (membrane-spanning) G protein coupled invention possessed seven hydrophobic domains. That is, it

derived cDNA fragment, and cloning of pG3-2 and pG1-10. amplification of a mouse pancreatic  $oldsymbol{eta}$  cell strain, MIN6 The present inventors have further succeeded in PCR

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Figure 27 has been derived. It was learned from the nucleotide CDNA is about 400 bp which is nearly comparable with the number with amino acid sequences [Figures 22 and 23] encoded by the G protein coupled receptor protein cDNA included in p19P2 cloned from the human pituitary gland. As a result, homology is more sequence, the presence of the third, fourth, fifth and sixth type G protein coupled receptor protein relative to the humansixth membrane-spanning domain of the known G protein coupled hydrophobicity plots [Figure 28]. The size of the amplified receptor protein. The amino acid sequence has been compared than 95% [Figure 61]. From this fact, it was estimated that the protein encoded by the cDNA included in pG3-2 is a mouse these two plasmid vectors, the nucleotide sequence shown in Upon translating the nucleotide sequence into an amino acid of bases between the third membrane-spanning domain and the Then, based on the nucleotide seguence of cDNA included in sequence that the cDNA encodes a novel receptor protein. membrane-spanning domains has been confirmed on the derived one encoded by the CDNA included in p19P2.

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confirmed on the hydrophobicity plots (Figure 64). The size of amino acid sequence (SEQ ID NO: 28), the presence of the third, The present inventors have further amplified a mouse protein cDNA included in p19P2 cloned from the human pituitary Figure 62 . From the nucleotide sequence (SEQ ID NO: 33), it with the known G protein coupled receptor protein. The amino [Figures 22 and 23] encoded by the G protein coupled receptor pancreatic eta -cell strain, MIN6-derived cDNA fragment by the PCR followed by subcloning into a plasmid vector to obtain a the amplified DNA is about 400 bp that is nearly comparable protein. Upon translating the nucleotide seguence into an fourth, fifth and sixth membrane-spanning domains has been gland, and with amino acid sequences of proteins encoded by pG3-2 and pG1-10 derived from the mouse pancreatic eta -cell acid sequence has been compared with amino acid sequences has been clarified that the cDNA encodes a novel receptor strain. As a result, homology is more than 95% to them clone (p5S38) having a nucleotide seguence as shown in

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eta -cell strain-derived p5S38, pertain to a receptor family that the human-derived pituitary gland-derived p1992, the proteins encoded by the mouse pancreatic  $\,eta\,$  -cell strain-derived pG3-2 [Figure 63]. This fact suggests that the protein encoded by and pG1-10, and the protein encoded by the mouse pancreatic recognizes the same ligand.

G protein coupled receptor protein, and use of said protein and Another object of the present invention is to provide protein coupled receptor protein, a process for producing said receptor protein, a DNA containing a DNA coding for said G a novel human amygdaloid nucleus-derived protein coupled

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receptor proteins, amplified an amygdaloid nucleus-derived cDNA for efficiently isolating a DNA coding for G protein coupled The present inventors have synthesized DNA primers with the above primer, and have analyzed it.

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its partial structure. The nucleotide sequence of the isolated isolating, from the human amygdaloid nucleus, a cDNA coding for From these facts, it is considered that the receptor protein is As a result, the present inventors have succeeded in human type GIR from the human amygdaloid nucleus. Accordingly, a novel G protein coupled receptor protein and have determined it is suggested that the isolated GIR is expressed even in the strongly expressed in the human brain and in the immune system The present inventors have succeeded in the isolation of this immunoregulating factors in the immune system on the T-cells. mouse glucocorticoid-induced receptor (hereinafter sometimes receptor protein to the same ligand (Molecular Endocrinology allow one to obtain a cDNA having a full length open reading 5:1331-1338, 1991). It is reputed that, in the mouse, the and is also functioning therein. These characterized DNAs referred to as "GIR") and is considered to be encoding a CDNA is preserved very well as compared with that of the GIR is a receptor which is induced by glucocorticoid and human central nervous system to carry out some function. expressed in T-cells and is working as a receptor to frame of the receptor and production of the receptor

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receptor protein-binding experiments, measurements of intracellular second messengers, etc. It further allows one non-natural compounds depending on indications obtainable in means, furthermore, permit screening for a ligand to the proteins. The receptor proteins expressed by a suitable between the ligand and the receptor protein. to screen for compounds capable of inhibiting the binding receptor proteins from the living body or from natural and

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hydrophobic clusters that exist in the G protein coupled one species, as shown in Figures 29 and 30, by PCR, cloned it, As a result, the presence of the fifth and fourth membraneside) and has been translated into an amino acid sequence. primer region at the 5' side (first membrane-spanning domain receptor proteins in common, i.e., corresponding to the first primers used for amplifying the cDNA are corresponding to seven that a novel receptor protein is encoded. The synthetic DNA and clarified from the analysis of a partial seguence thereof amplified, as a novel human amygdaloid nucleus-derived cDNA, of the known G protein coupled receptor protein. plots [Figure 32]. The size of the amplified cDNA is about spanning domains has been confirmed on the hydrophobicity side) and has been translated into an amino acid sequence . primer region at the 3' side (sixth membrane-spanning domain Similarly, the nucleotide sequence has been determined from the have been confirmed on the hydrophobicity plotting [Figure 31]. As a result, the second and third membrane-spanning domains domains. The nucleotide sequence has been determined from the and sixth membrane-spanning regions among the membrane-spanning 700 bp which is nearly comparable with the number of bases To be more specific, the present inventors have

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is a widely known G protein coupled receptor protein [Figure 33]. This result strongly suggests that the DNA of the present for mouse-derived glucocorticoid-induced receptor protein which isolated DNA and observed high homology to the DNA that codes invention is encoding a human-type receptor protein of GIR based on, as a template, the nucleotide sequence of the The inventors have further retrieved the data base

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10 of said protein and DNA. The present inventors have primer, and have analyzed it. pancreatic eta -cell strain, MIN6-derived cDNA with the above synthesized DNA primers for efficiently isolating a DNA coding for producing said G protein coupled receptor protein, and use coding for said G protein coupled receptor protein, a process protein coupled receptor protein, a DNA containing a DNA for G protein coupled receptor proteins, amplified a mouse provide a novel mouse pancreatic  $oldsymbol{eta}$  -cell strain, MIN6-derived Yet another object of the present invention is to

allow one to obtain a cDNA having a full length open reading expressed by a suitable means, furthermore, permit screening frame of the receptor and production of the receptor novel receptor protein which is expressed in the mouse pancreas coupled receptors at the nucleotide sequence level and at the coupled receptor protein and have determined its partial inhibiting the binding of the ligand with the receptor protein. It further allows one to screen for compounds capable of measurements of intracellular second messengers, etc. obtainable in receptor protein-binding experiments, from natural and non-natural compounds relying on indications for a ligand to the receptor protein from the living body or probe, said mouse-derived cDNA. The receptor proteins proteins. Human-derived cDNAs may be cloned by using, as a and is also functioning therein. These characterized DNAs amino acid sequence level and is considered to be encoding a structure. The isolated cDNA is homologous to known G protein isolating a mouse-derived cDNA coding for a novel G protein As a result, the present inventors have succeeded in

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35 fifth and sixth membrane-spanning domains has been confirmed nucleotide sequence has been translated into an amino acid it, and clarified from the analysis of a partial sequence derived cDNA, p3H2-17, as shown in Figures 37, by PCR, cloned amplified, as a novel mouse pancreatic  $\beta$  -cell strain, MIN6sequence. As a result, the presence of the third, fourth, thereof that a novel receptor protein is encoded. The To be more specific, the present inventors have

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on the hydrophobicity plots (Figure 38). The size of the amplified CDNA is about 400 bp which is nearly comparable with that of the known G protein coupled receptor protein.

The inventors have retrieved the data base based on, as a template, the nucleotide sequence of the isolated DNA and observed 30% homology to chicken ATP receptor (P34996), 25% homology to human somatostatin receptor subtype 3 (A46226), and 28% homology to human somatostatin receptor subtype 4 (JN0605), and 28% homology to bovine neuropeptide Y receptor (S28787), respectively (Figure 39), which are known G protein coupled receptor proteins. The aforementioned abbreviations in parentheses are reference numbers that are assigned when they are registered as data to NBRF-PIR/Swiss-PROT and are, usually, each called "Accession Number".

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An expression of receptor genes encoded by the CDNA fragment included in p3H2-17 of the present invention has been checked by northern blotting techniques at a mRNA level, and it has been confirmed that the receptor gene has been intensely expressed in the mouse thymus and spleen. It has been also confirmed that the receptor gene has been expressed in the mouse brain and pancreas (Figure 65).

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Next, by utilizing the information on the nucleotide sequence of the fragment included in p3H2-17, cDNA encoding a full-length open reading frame of the mouse pancreatic \$\beta\$ -cell strain, MIN6-derived G protein coupled receptor protein of the present invention has been obtained from mouse thymic and spleenic poly(A) \*RNA by 5'RACE (5' rapid amplification of cDNA ends) techniques (Frohman M.A. et al., Proc. Natl. Acad. Sci. USA, 85:8998-9002 (1988); Belyavsky A. et al., Nucleic Acids Res., 17:2919-2932 (1989); Edwards J.B.D.M. et al., Nucleic Acids Res., 19:5227-5232 (1991)) and 3'RACE (3' rapid amplification of cDNA ends) techniques (Frohman M.A. et al., Proc. Natl. Acad. Sci. USA, 85:8998-9002 (1988); Belyavsky A. et al., Nucleic Acids Res., 17:2919-2932 (1989)).

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The plasmid (pMAH2-17) carrying cDNA encoding a full-length open reading frame of the receptor protein of the

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present invention has been subjected to sequencing analysis. As a result, the nucleotide sequence of the region coding for the receptor protein is represented by SEQ ID NO: 41 and the amino acid sequence deduced therefrom is represented by SEQ ID NO: 39 (Figure 69). Based on the amino acid sequence, hydrophobicity plotting has been carried out. The results are shown in Figure 70.

It has been clarified from the hydrophobicity plotting that the mouse pancreatic  $\beta$  -cell strain, MIN6-derived receptor protein of the present invention has seven hydrophobic domains. Thus, it has been confirmed that the receptor protein encoded by the CDNA included in pMAH2-17 according to the present invention is a seven transmembrane G protein coupled receptor protein.

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Data base retrieval has been carried out based on the full-length amino acid sequence encoded by the CDNA included in pMAH2-17, and it has been observed that the amino acid sequence has 44.0% homology to mouse P\_20 purinoceptor (P35383) and 38.1% homology to chicken P\_2x purinoceptor (P34996), respectively (Figure 71), which are known G protein coupled receptor proteins. The aforementioned abbreviations in parentheses are reference numbers that are assigned when they are registered as data to NBRF-PIR/Swiss-PROT and are, usually, each called "Accession Number". Since the receptor protein encoded by pMAH2-17 is highly homologous to prinoceptors, it is considered that there are strong possibility of a subtype within prinoceptor families. Therefore, the present inventors have carried out an

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Occytes and found significant inward currents elicited by <u>Xenopus</u> occytes carrying the subject receptor gene in response to ATP stimulation (Figure 75). As a result, it has been determined that the receptor encoded by pMAH2-17 is one of the subtypes within prinoceptor families. It has been discussed and expected that there are a variety of subtypes among purinoceptors (Pharmac. Ther., Vol. 64, pp. 445-475 (1994).

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electrophysiological analysis of the receptor gene in Xenopus

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Natl. Acad. Sci. USA, Vol. 91, pp.3275-3279 (1994)); and 12, pp. 27-32 (1995)); human  $P_{2u}$  or  $P_{2y2}$  purinoceptor (Proc. Vol. 324(2), 219-225 (1993)); mouse  $P_{2y2}$  or  $P_{2u}$  purinoceptor clearly distinct from chicken P  $_{2\mathrm{y1}}$  purinoceptor (FEBS LETTERS. encoded by pMAH2-17) is a novel purinoceptor subtype which is invention (e.g., SEQ ID NO: 38 and SEQ ID NO: 39, or proteins rat P<sub>2x</sub> purinoceptor (Nature, Vol. 371.6, pp.516-519 (1994).  $m P_{2u}$  or  $m P_{2y2}$  purinoceptor (Am. J. Respir. Cell Mol. Biol., Vol. (Proc. Natl. Acad. Sci. USA, Vol. 90, pp.5113-5117 (1993)); rat eta -cell strain, MIN6-derived receptor protein of the present It is also strongly suggested that agonists and/or All data are supporting that the mouse pancreatic

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diseases or syndromes in connection with purine ligand or prophylactically treating hypertension, diabetes, cystic encoded by pMAH2-17 are useful as an immunomodulator or an compounds. It is expected that the agonists of the receptor would be useful in therapeutic or prophylactic treatment of antagonists related to the receptor encoded by pMAH2-17 the receptor encoded by pMAH2-17 are useful as hypotensive fibrosis, etc. It is still expected that the antagonists of antitumor agent, in addition they are useful in therapeutically prophylactically treating incontinence of urine, etc. agents, analgesics, agents for therapeutically or

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30 of prinoceptor type, a DNA containing a DNA coding for said G efficiently isolating a DNA coding for prinoceptor type G G protein coupled receptor protein, and use of said protein and provide a novel human-derived protein coupled receptor protein derived cDNA with the above primer, and have analyzed it. nucleotide sequence of mouse purinoceptor, amplified a humanprotein coupled receptor proteins on the basis of the DNA. The present inventors have synthesized DNA primers for protein coupled receptor protein, a process for producing said Another object of the present invention is to

isolating a human-derived cDNA coding for a novel G protein coupled receptor protein and have determined its full-length structure [Figure 77]. The isolated cDNA is homologous to As a result, the present inventors have succeeded in

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10 a suitable means, furthermore, permit screening for a ligand to non-natural compounds relying on indications obtainable in novel purinoceptor protein. The receptor proteins expressed by (87% homology; Figure 79) and is considered to be encoding a of the ligand with the receptor protein. one to screen for compounds capable of inhibiting the binding receptor protein-binding experiments, etc. It further allows the receptor protein from the living body or from natural and nucleotide sequence level and at the amino acid sequence level mouse G protein coupled receptor (purinoceptor) at the

compounds. It is expected that the agonists of the human would be useful in therapeutic or prophylactic treatment of agent, in addition they are useful in therapeutically or diseases or syndromes in connection with purine ligand antagonists related to the human receptor encoded by phAH2-17 treating incontinence of urine, etc. analgesics, agents for therapeutically or prophylactically the human receptor are useful as hypotensive agents, fibrosis, etc. It is still expected that the antagonists of prophylactically treating hypertension, diabetes, cystic receptor are useful as an immunomodulator or an antitumor It is also strongly suggested that agonists and/or

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Accordingly, one aspect of the present invention is

- (1) DNAs comprising a nucleotide sequence
- 25 of SEQ ID NO: 1 to SEQ ID NO: 19; represented by a SEQ ID NO selected from the group consisting
- the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9; nucleotide sequence represented by a SEQ ID NO selected from (2) DNAs according to the above (1) comprising a
- nucleotide sequence represented by SEQ ID NO: 1 or SEQ ID (3) DNAs according to the above (1) comprising a

- a DNA coding for a G protein coupled receptor protein; is a primer for polymerase chain reaction in order to amplify (4) DNAs according to the above (1) wherein the DNA
- (5) a method for amplifying a DNA coding for a G

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protein coupled receptor protein by polymerase chain reaction techniques, which comprises:

- (i) carrying out a polymerase chain reaction in the presence of a mixture of
- a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,

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at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 5, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 7, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 14, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 14, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 16 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 18, and

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at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19; or

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(ii) carrying out a polymerase chain reaction in the presence of a mixture of

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a DNA coding for G protein coupled receptor protein,

said DNA being capable of acting as a template,

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- © at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 13;
- (6) a method for screening a DNA library for 10 a DNA coding for a G protein coupled receptor protein, which comprises:
- (i) carrying out a polymerase chain reaction in the presence of a mixture of
- Said DNA library,

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at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 5, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 7, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 14, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 16 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 16 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 18, and

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© at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11, DNA primers comprising a nucleotide

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comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19, sequence represented by SEQ ID NO: 15, DNA primers

coupled receptor protein, contained in the DNA library; or of a mixture of to amplify selectively a template DNA coding for G protein carrying out a polymerase chain reaction in the presence

- said DNA library
- 0 at least one DNA primer selected from the group sequence represented by SEQ ID NO: 1 and DNA primers consisting of DNA primers comprising a nucleotide comprising a nucleotide sequence represented by SEQ ID NO: 12, and
- 15 receptor protein, contained in the DNA library; to amplify selectively a DNA coding for G protein coupled Θ at least one DNA primer selected from the group sequence represented by SEQ ID NO: 13, consisting of DNA primers comprising a nucleotide
- protein, which is obtained by a method according to the above (5) or (6); and 3 a DNA coding for a G protein coupled receptor

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a DNA according to the above (7), their peptide segments or fragments and salts thereof. (8) G protein coupled receptor proteins encoded by

Another specific aspect of the invention is:

polymerase chain reaction techniques, which comprises carrying protein coupled receptor proteins or other domains thereof) by protein coupled receptor protein (e.g. a region corresponding to from the first to sixth membrane-spanning domains of G (9) a method for amplifying a DNA coding for G

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- out a polymerase chain reaction in the presence of a mixture of a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
- 0 at least one DNA primer selected from the group sequence represented by SEQ ID NO: 1 and DNA primers consisting of DNA primers comprising a nucleotide

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comprising a nucleotide sequence represented by SEQ ID NO: 12, and

❷ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide SEQ ID NO: 15, DNA primers comprising a nucleotide primers comprising a nucleotide sequence represented by nucleotide sequence represented by SEQ ID NO: 9, DNA represented by SEQ ID NO: 8, DNA primers comprising a NO: 4, DNA primers comprising a nucleotide sequence comprising a nucleotide sequence represented by SEQ ID sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID sequence represented by SEQ ID NO: 17 and DNA primers

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- 15 20 out a polymerase chain reaction in the presence of a mixture of polymerase chain reaction techniques, which comprises carrying protein coupled receptor proteins or other domains thereof) by to from the first to seventh membrane-spanning domains of G protein coupled receptor protein (e.g. a region corresponding a DNA coding for G protein coupled receptor protein, (10) a method for amplifying a DNA coding for G
- 0 at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide comprising a nucleotide sequence represented by SEQ ID sequence represented by SEQ ID NO: 1 and DNA primers

said DNA being capable of acting as a template,

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Θ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11;

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protein coupled receptor proteins or other domains thereof) by protein coupled receptor protein (e.g. a region corresponding polymerase chain reaction techniques, which comprises carrying to from the third to sixth membrane-spanning domains of G out a polymerase chain reaction in the presence of a mixture of a DNA coding for G protein coupled receptor protein, (11) a method for amplifying a DNA coding for G

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primers comprising a nucleotide seguence represented by comprising a nucleotide sequence represented by SEQ ID represented by SEQ ID NO: 6, DNA primers comprising a SEQ ID NO: 14 and DNA primers comprising a nucleotide nucleotide sequence represented by SEQ ID NO: 7, DNA NO: 5, DNA primers comprising a nucleotide sequence sequence represented by SEQ ID NO: 3, DNA primers consisting of DNA primers comprising a nucleotide at least one DNA primer selected from the group said DNA being capable of acting as a template, seguence represented by SEQ ID NO: 18, and 0

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primers comprising a nucleotide sequence represented by comprising a nucleotide sequence represented by SEQ ID comprising a nucleotide sequence represented by SEQ ID represented by SEQ ID NO: 8, DNA primers comprising a sequence represented by SEQ ID NO: 17 and DNA primers nucleotide sequence represented by SEQ ID NO: 9, DNA NO: 4, DNA primers comprising a nucleotide sequence SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers consisting of DNA primers comprising a nucleotide at least one DNA primer selected from the group NO: 19; 0

out a polymerase chain reaction in the presence of a mixture of protein coupled receptor proteins or other domains thereof) by polymerase chain reaction techniques, which comprises carrying protein coupled receptor protein (e.g. a region corresponding a DNA coding for G protein coupled receptor protein, to from the third to seventh membrane-spanning domains of G (12) a method for amplifying a DNA coding for G consisting of DNA primers comprising a nucleotide at least one DNA primer selected from the group said DNA being capable of acting as a template, Θ 0

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comprising a nucleotide sequence represented by SEQ ID represented by SEQ ID NO: 6, DNA primers comprising a NO: 5, DNA primers comprising a nucleotide sequence sequence represented by SEQ ID NO: 3, DNA primers

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primers comprising a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers comprising a nucleotide nucleotide sequence represented by SEQ ID NO: 7, DNA sequence represented by SEQ ID NO: 18, and

consisting of DNA primers comprising a nucleotide at least one DNA primer selected from the group sequence represented by SEQ ID NO: 11; 0

out a polymerase chain reaction in the presence of a mixture of protein coupled receptor proteins or other domains thereof) by polymerase chain reaction techniques, which comprises carrying protein coupled receptor protein (e.g. a region corresponding to from the second to sixth membrane-spanning domains of G (13) a method for amplifying a DNA coding for G

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a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,

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comprising a nucleotide sequence represented by SEQ ID sequence represented by SEQ ID NO: 10 and DNA primers consisting of DNA primers comprising a nucleotide at least one DNA primer selected from the group NO: 16, and 0

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primers comprising a nucleotide sequence represented by comprising a nucleotide sequence represented by SEQ ID represented by SEQ ID NO: 8, DNA primers comprising a sequence represented by SEQ ID NO: 17 and DNA primers nucleotide sequence represented by SEQ ID NO: 9, DNA NO: 4, DNA primers comprising a nucleotide seguence comprising a nucleotide sequence represented by SEQ SEQ ID NO: 15, DNA primers comprising a nucleotide consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers at least one DNA primer selected from the group NO: 19; 0

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protein coupled receptor proteins or other domains thereof) by polymerase chain reaction techniques, which comprises carrying protein coupled receptor protein (e.g. a region corresponding to from the second to seventh membrane-spanning domains of G (14) a method for amplifying a DNA coding for G

out a polymerase chain reaction in the presence of a mixture of a DNA coding for G protein coupled receptor protein,

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0 at least one DNA primer selected from the group said DNA being capable of acting as a template, comprising a nucleotide sequence represented by SEQ ID consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10 and DNA primers NO: 16, and

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- Θ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide
- protein coupled receptor proteins or other domains thereof) by protein coupled receptor protein (e.g. a region corresponding out a polymerase chain reaction in the presence of a mixture of polymerase chain reaction techniques, which comprises carrying to from the first to third membrane-spanning domains of G sequence represented by SEQ ID NO: 11; (15) a method for amplifying a DNA coding for G

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a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,

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- 0 at least one DNA primer selected from the group primers comprising a nucleotide sequence sequence represented by SEQ ID NO: 1 and DNA consisting of DNA primers comprising a nucleotide represented by SEQ ID NO: 12, and
- Θ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 13; (16) a method for amplifying a DNA coding for G
- techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of protein coupled receptor protein by polymerase chain reaction

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- a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
- 0 at least one DNA primer selected from the group sequence represented by SEQ ID NO: 1, and consisting of DNA primers comprising a nucleotide

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at least one DNA primer selected from the group

consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2;

- Ç techniques, which comprises carrying out a polymerase chain protein coupled receptor protein by polymerase chain reaction reaction in the presence of a mixture of (17) a method for amplifying a DNA coding for G
- a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
- at least one DNA primer selected from the group sequence represented by SEQ ID NO: 3, and consisting of DNA primers comprising a nucleotide

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- consisting of DNA primers comprising a nucleotide at least one DNA primer selected from the group sequence represented by SEQ ID NO: 4; (18) a method for amplifying a DNA coding for G
- protein coupled receptor protein by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

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a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,

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- at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, and
- Θ at least one DNA primer selected from the group sequence represented by SEQ ID NO: 8; consisting of DNA primers comprising a nucleotide (19) a method for amplifying a DNA coding for G

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- reaction in the presence of a mixture of protein coupled receptor protein by polymerase chain reaction techniques, which comprises carrying out a polymerase chain
- a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,

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- at least one DNA primer selected from the group sequence represented by SEQ ID NO: 10, and consisting of DNA primers comprising a nucleotide
- at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide

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sequence represented by SEQ ID NO: 11;

carrying out a polymerase chain reaction in the presence (20) a method for amplifying DNA coding for a G protein coupled receptor protein which comprises

a DNA coding for G protein coupled receptor protein, of a mixture of

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- said DNA being capable of acting as a template,
- primers comprising a nucleotide sequence represented by comprising a nucleotide sequence represented by SEQ ID represented by SEQ ID NO: 3, DNA primers comprising a nucleotide seguence represented by SEQ ID NO: 5, DNA NO: 1, DNA primers comprising a nucleotide seguence selected from the group consisting of DNA primers SEQ ID NO: 6, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 7, DNA primers coding for G protein coupled receptor protein to in the 5' o 3' direction, said DNA primer being nucleotide sequence represented by SEQ ID NO: 12, nucleotide sequence represented by SEQ ID NO: 18, binding with the 3'-side nucleotide seguence of allow the extension of the + chain (plus chain) comprising a nucleotide sequence represented by comprising a nucleotide sequence represented by the - chain (minus chain) of the template DNA at least one DNA primer which is capable of DNA primers comprising a nucleotide sequence SEQ ID NO: 16 and DNA primers comprising a represented by SEQ ID NO: 14, DNA primers SEQ ID NO: 10, DNA primers comprising a 0

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selected from the group consisting of DNA primers coding for G protein coupled receptor protein to in the 5' - 3' direction, said DNA primer being allow the extension of the - chain (minus chain) binding with the 3'-side nucleotide seguence of the + chain (plus chain) of the template DNA at least one DNA primer which is capable of 0

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primers comprising a nucleotide sequence represented by comprising a nucleotide sequence represented by SEQ ID represented by SEQ ID NO: 4, DNA primers comprising a nucleotide seguence represented by SEQ ID NO: 8, DNA NO: 2, DNA primers comprising a nucleotide seguence SEQ ID NO: 9, DNA primers comprising a nucleotide nucleotide seguence represented by SEQ ID NO: 19, comprising a nucleotide sequence represented by sequence represented by SEQ ID NO: 11, DNA SEQ ID NO: 17 and DNA primers comprising a represented by SEQ ID NO: 15, DNA primers primers comprising a nucleotide seguence

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carrying out a polymerase chain reaction in the presence of a mixture of (ii)

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a DNA coding for G protein coupled receptor protein,

said DNA being capable of acting as a template,

NO: 1 and DNA primers comprising a nucleotide seguence comprising a nucleotide sequence represented by SEQ IN selected from the group consisting of DNA primers coding for G protein coupled receptor protein to in the 5' o 3' direction, said DNA primer being allow the extension of the + chain (plus chain) binding with the 3'-side nucleotide seguence of the - chain (minus chain) of the template DNA at least one DNA primer which is capable of represented by SEQ ID NO: 12, and 0

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comprising a nucleotide sequence represented by SEQ ID selected from the group consisting of DNA primers coding for G protein coupled receptor protein to allow the extension of the - chain (minus chain) in the 5'  $\rightarrow$  3' direction, said DNA primer being binding with the 3'-side nucleotide sequence of the + chain (plus chain) of the template DNA at least one DNA primer which is capable of 0

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a method for screening DNA libraries for

of G protein coupled receptor protein), which comprises the first to sixth membrane-spanning domains or other domains a DNA coding for G protein coupled receptor protein (e.g. from mixture of carrying out a polymerase chain reaction in the presence of a

said DNA library,

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- at least one DNA primer selected from the group represented by SEQ ID NO: 12, and primers comprising a nucleotide seguence sequence represented by SEQ ID NO: 1 and DNA consisting of DNA primers comprising a nucleotide
- **@** at least one DNA primer selected from the group sequence represented by SEQ ID NO: 2, DNA primers consisting of DNA primers comprising a nucleotide comprising a nucleotide sequence represented by sequence represented by SEQ ID NO: 8, DNA primers SEQ ID NO: 4, DNA primers comprising a nucleotide comprising a nucleotide sequence represented by represented by SEQ ID NO: 17 and DNA primers primers comprising a nucleotide sequence sequence represented by SEQ ID NO: 15, DNA SEQ ID NO: 9, DNA primers comprising a nucleotide comprising a nucleotide sequence represented by SEQ ID NO: 19,

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coupled receptor protein (e.g. from the first to sixth to amplify selectively a template DNA coding for G protein membrane-spanning domains or other domains of G protein coupled receptor protein), contained in the DNA library;

- ä a DNA coding for G protein coupled receptor protein (e.g. from of G protein coupled receptor protein), which comprises the first to seventh membrane-spanning domains or other domains mixture of carrying out a polymerase chain reaction in the presence of a (22) a method for screening DNA libraries for
- said DNA library,

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 ${\mathbb Q}$  at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide

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comprising a nucleotide sequence represented by SEQ ID sequence represented by SEQ ID NO: 1 and DNA primers

at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11, NO: 12, and

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coupled receptor protein (e.g. from the first to seventh to amplify selectively a template DNA coding for G protein receptor protein), contained in the DNA library; membrane-spanning domains or other domains of G protein coupled

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- a DNA coding for G protein coupled receptor protein (e.g. from carrying out a polymerase chain reaction in the presence of a of G protein coupled receptor protein), which comprises the third to sixth membrane-spanning domains or other domains mixture of (23) a method for screening DNA libraries for
- said DNA library,

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- at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, DNA primers SEQ ID NO: 5, DNA primers comprising a nucleotide comprising a nucleotide sequence represented by sequence represented by SEQ ID NO: 3, DNA primers comprising a nucleotide sequence represented by primers comprising a nucleotide sequence sequence represented by SEQ ID NO: 14 and DNA SEQ ID NO: 7, DNA primers comprising a nucleotide represented by SEQ ID NO: 18, and
- 0 at least one DNA primer selected from the group comprising a nucleotide sequence represented by sequence represented by SEQ ID NO: 2, DNA primers consisting of DNA primers comprising a nucleotide comprising a nucleotide sequence represented by sequence represented by SEQ ID NO: 8, DNA primers SEQ ID NO: 4, DNA primers comprising a nucleotide SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 15, DNA

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primers comprising a nucleotide sequence

comprising a nucleotide sequence represented by represented by SEQ ID NO: 17 and DNA primers

SEQ ID NO: 19,

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membrane-spanning domains or other domains of G protein coupled to amplify selectively a template DNA coding for G protein coupled receptor protein (e.g. from the third to sixth receptor protein), contained in the DNA library;

the third to seventh membrane-spanning domains or other domains a DNA coding for G protein coupled receptor protein (e.g. from carrying out a polymerase chain reaction in the presence of a (24) a method for screening DNA libraries for of G protein coupled receptor protein), which comprises mixture of

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said DNA library,

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consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, DNA primers SEQ ID NO: 5, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, DNA primers SEQ ID NO: 7, DNA primers comprising a nucleotide at least one DNA primer selected from the group comprising a nucleotide sequence represented by comprising a nucleotide sequence represented by seguence represented by SEQ ID NO: 14 and DNA Primers comprising a nucleotide sequence represented by SEQ ID NO: 18, and

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- consisting of DNA primers comprising a nucleotide at least one DNA primer selected from the group sequence represented by SEQ ID NO: 11, 0
- membrane-spanning domains or other domains of G protein coupled to amplify selectively a template DNA coding for G protein coupled receptor protein (e.g. from the third to seventh receptor protein), contained in the DNA library;

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a DNA coding for G protein coupled receptor protein (e.g. from the second to sixth membrane-spanning domains or other domains (25) a method for screening DNA libraries for of G protein coupled receptor protein), which comprises 35

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carrying out a polymerase chain reaction in the presence of a mixture of

said DNA library,

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consisting of DNA primers comprising a nucleotide at least one DNA primer selected from the group sequence represented by SEQ ID NO: 10 and DNA primers comprising a nucleotide seguence represented by SEQ ID NO: 16, and

sequence represented by SEQ ID NO: 2, DNA primers consisting of DNA primers comprising a nucleotide SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers SEQ ID NO: 9, DNA primers comprising a nucleotide at least one DNA primer selected from the group comprising a nucleotide sequence represented by comprising a nucleotide sequence represented by comprising a nucleotide sequence represented by represented by SEQ ID NO: 17 and DNA primers sequence represented by SEQ ID NO: 15, DNA Primers comprising a nucleotide seguence SEQ ID NO: 19, 0

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membrane-spanning domains or other domains of G protein couply to amplify selectively a template DNA coding for G protein coupled receptor protein (e.g. from the second to sixth

domains of G protein coupled receptor protein), which comprises a DNA coding for G protein coupled receptor protein (e.g. from carrying out a polymerase chain reaction in the presence of a (26) a method for screening DNA libraries for the second to seventh membrane-spanning domains or other receptor protein), contained in the DNA library; mixture of

- said DNA library,
- consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10 and DNA Primers comprising a nucleotide seguence represented by SEQ ID NO: 16, and

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at least one DNA primer selected from the group

Θ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11,

coupled receptor protein (e.g. from the second to seventh to amplify selectively a template DNA coding for G protein receptor protein), contained in the DNA library; membrane-spanning domains or other domains of G protein coupled

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- the first to third membrane-spanning domains or other domains a DNA coding for G protein coupled receptor protein (e.g. from of G protein coupled receptor protein), which comprises mixture of carrying out a polymerase chain reaction in the presence of a (27) a method for screening DNA libraries for
- said DNA library,

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- 0 at least one DNA primer selected from the group NO: 12, and comprising a nucleotide sequence represented by SEQ ID sequence represented by SEQ ID NO: 1 and DNA primers consisting of DNA primers comprising a nucleotide
- Θ consisting of DNA primers comprising a nucleotide at least one DNA primer selected from the group sequence represented by SEQ ID NO: 13,

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receptor protein), contained in the DNA library; membrane-spanning domains or other domains of G protein coupled coupled receptor protein (e.g. from the first to third to amplify selectively a template DNA coding for G protein

- presence of a mixture of comprises carrying out a polymerase chain reaction in the a DNA coding for G protein coupled receptor protein, which (28) a method for screening DNA libraries for
- said DNA library,

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- at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1, and
- Θ at least one DNA primer selected from the group sequence represented by SEQ ID NO: 2, consisting of DNA primers comprising a nucleotide

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coupled receptor protein, contained in the DNA library; to amplify selectively the template DNA coding for G protein

- S presence of a mixture of comprises carrying out a polymerase chain reaction in the DNA coding for G protein coupled receptor protein, which (29) a method for screening DNA libraries to detect
- said DNA library,
- at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, and

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Θ at least one DNA primer selected from the group sequence represented by SEQ ID NO: 4, consisting of DNA primers comprising a nucleotide

15 a DNA coding for G protein coupled receptor protein, which presence of a mixture of coupled receptor protein, contained in the DNA library; comprises carrying out a polymerase chain reaction in the to amplify selectively a template DNA coding for G protein (30) a method for screening DNA libraries for

- 20 said DNA library,
- at least one DNA primer selected from the group sequence represented by SEQ ID NO: 6, and consisting of DNA primers comprising a nucleotide
- at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8,

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coupled receptor protein, contained in the DNA library; to amplify selectively a template DNA coding for G protein

presence of a mixture of comprises carrying out a polymerase chain reaction in the a DNA coding for G protein coupled receptor protein, which (31) a method for screening DNA libraries for

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- said DNA library,
- at least one DNA primer selected from the group sequence represented by SEQ ID NO: 10, and consisting of DNA primers comprising a nucleotide

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0 at least one DNA primer selected from the group

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consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11,

to amplify selectively a template DNA coding for G protein coupled receptor protein, contained in the DNA library; and

liver, lymph gland, lung, thymus, placenta, peritoneum, retina, a method for screening DNA libraries according consisting of human tissues and human cells. Examples of such spleen, heart, smooth muscle, intestine, vessel, bone, kidney, human tissues include adrenal, umbilical cord, brain, tongue, pancreas, submandibular gland, spine, prostate gland, stomach, adipose tissue, urinary bladder, cornea, olfactory bulb, bone nerve cells, epithelial cells, endothelial cells, leukocytes, skin, fetus, mammary gland, ovary, testis, pituitary gland, thyroid gland, trachea (windpipe), skeletal muscle, uterus, to any of the above (6), and (21) to (31) wherein said DNA marrow, amnion, etc. Examples of such human cells include library is derived from an origin selected from the group osteoblasts, osteoclasts, astrocytes, melanocytes, various carcinomas, various sarcomas, various cells derived from lymphocytes, gliacytes, fibroblasts, keratinized cells, the above-mentioned human tissues. (35)

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Yet another aspect of the present invention is a degenerate deoxynucleotide which has an oligonucleotide sequence to which a SEQ ID NO selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 19 is assigned.

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Another aspect of the present invention is

an amino acid sequence selected from the group consisting of amino acid sequences represented by SEQ ID NO: 24 and/or SEQ ID NO: 25 and substantial equivalents to the amino acid sequence represented by SEQ ID NO: 24 or SEQ ID NO: 25; or a salt thereof;

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(34) a G protein coupled receptor protein according to the above (33) comprising an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 26 and substantial equivalents to the amino

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acid sequence represented by SEQ ID NO: 26; or a salt thereof;

an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 27 and substantial equivalents to the amino acid sequence represented by SEQ ID NO: 27; or a salt thereof;

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(36) a G protein coupled receptor protein comprising an amino acid sequence selected from the group consisting of amino acid sequence represented by SEQ ID NO: 28 and substantial equivalents to the amino acid sequence

epresented by SEQ ID NO: 28; or a salt thereof;

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an amino acid sequence selected from the group consisting of amino acid sequences represented by SEQ ID NO: 34 and/or SEQ ID NO: 35 and substantial equivalents to the amino acid sequence represented by SEQ ID NO: 35 or a salt thereof;

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(38) a G protein coupled receptor protein comprising an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 38 and substantial equivalents to the amino acid sequence

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represented by SEQ ID NO: 38; or a salt thereof;

(39) a G protein coupled receptor protein according to the above (38) comprising an amino acid sequence selected from the group consisting of an amino acid sequence represent by SEQ ID NO: 39 and substantial equivalents to the amino acid sequence represented by SEQ ID NO: 39; or a salt thereof;

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(40) a G protein coupled receptor protein comprising an amino acid sequence represented by SEQ ID NO: 56 and

substantial equivalents to the amino acid sequence represented by SEQ ID NO: 56; or a salt thereof;

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(41) a peptide segment or fragment of a G protein coupled receptor protein according to any of the above (33) to (40), a modified derivative thereof or a salt thereof;

. (42) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (33);

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(43) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (34);

(44) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (35);
(45) a DNA which comprises a nucleotide sequence

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- coding for a G protein coupled receptor protein of the above (36);

  (46) a DNA which comprises a nucleotide sequence coding for a G protein coupled recentor protein of the above
- coding for a G protein coupled receptor protein of the above (37);

  (47) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (38);
- (48) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (39);

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(49) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (40);

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- (50) a DNA of the above (42) comprising a nucleotide sequence represented by SEQ ID NO: 29 and/or SEQ ID NO: 30;
- (51) a DNA of the above (43) comprising a nucleotide sequence represented by SEQ ID NO: 31;
- (52) a DNA of the above (44) comprising a nucleotide sequence represented by SEQ ID NO: 32;
- (53) a DNA of the above (45) comprising a nucleotide sequence represented by SEQ ID NO: 33;
- (54) a DNA of the above (46) comprising a nucleotide sequence represented by SEQ ID NO: 36 and/or SEQ ID NO: 37;

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- (55) a DNA of the above (47) comprising a nucleotide sequence represented by SEQ ID NO: 40;
- (56) a DNA of the above (48) comprising a nucleotide sequence represented by SEQ  $\cdot$ ID NO: 41;

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(57) a DNA of the above (49) comprising a nucleotide sequence represented by SEQ ID NO: 57;

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- (58) a vector comprising a DNA according to any of the above (42) to (57);
- (59) a transformant (including a transfectant)
  carrying a vector of the above (58);
- 5 (60) a process for producing a G protein coupled receptor protein or a salt thereof according to any of the above (33) to (40), which comprises culturing a transformant of the above (59) to express said G protein coupled receptor protein on the membrane of the transformant;
- 10 (61) a method for determining a ligand to a G protein coupled receptor protein according to any of the above (33) to (40), which comprises contacting
- (i) at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof, with

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(ii) at least one compound to be tested;

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(62) a screening method for a compound capable of inhibiting the binding of a G protein coupled receptor protein according to any of the above (33) to (40) with a ligand, which comprises carrying out a comparison between:

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(i) at least one case where said ligand is contacted with at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof, and

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(ii) at least one case where said ligand together with a compound to be tested is contacted with at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the

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above (41), and mixtures thereof;

compounds capable of inhibiting the binding of a G protein coupled receptor protein according to any of the above (33) to (40), with a ligand, which comprises at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to salts thereof according to the above (41), and mixtures thereof; and

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selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof.

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Yet another aspect of the present invention is (65) a G protein coupled receptor protein according to the above (33) comprising

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of an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 24, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 24, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 one or more amino acid residues (preferably from 2 to 30

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amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 24, and amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 24 are substituted with one or more other amino acid residues, or/and

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(ii) an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 25, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 25, amino acid

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sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 25, and amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 25 are substituted with one or more other amino acid residues, or a salt thereof;

to the above (34) comprising an amino acid sequence selected from the group consisting of an amino acid sequence selected by SEQ ID NO: 26, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 26, amino acid sequences wherein one or more amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 26, amino acid sequences wherein one or more amino acid residues. (preferably from 2 to 30 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 26, and amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues) in the amino acid sequence of SEQ ID NO: 26 are substituted with one or more other amino acid residues, or a salt thereof;

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to the above (35) comprising an amino acid sequence selected from the group consisting of an amino acid sequence selected by SEQ ID NO: 27, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 27, amino acid sequences wherein one or more amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 27, and amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues) in residues, more preferably from 2 to 10 amino acid residues) in

one or more other amino acid residues, or a salt thereof; the amino acid sequence of SEQ ID NO: 27 are substituted with

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- deleted from the amino acid sequence of SEQ ID NO: 28, amino amino acid residues (preferably from 2 to 30 amino acid from the group consisting of an amino acid sequence represented to the above (36) comprising an amino acid sequence selected residues, more preferably from 2 to 10 amino acid residues) in or more amino acid residues (preferably from 2 to 30 amino acid sequence of SEQ ID NO: 28, and amino acid sequences wherein one from 2 to 10 amino acid residues) are added to the amino acid acid sequences wherein one or more amino acid residues residues, more preferably from 2 to 10 amino acid residues) are by SEQ ID NO: 28, amino acid sequences wherein one or more one or more other amino acid residues, or a salt thereof; the amino acid sequence of SEQ ID NO: 28 are substituted with (preferably from 2 to 30 amino acid residues, more preferably (68) a G protein coupled receptor protein according
- to the above (37) comprising a G protein coupled receptor protein according

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(i) an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 34, amino acid sequences wherein one or more amino acid residues amino acid residues, more preferably from 2 to 10 amino acid one or more amino acid residues (preferably from 2 to 30 acid sequence of SEQ ID NO: 34, amino acid sequences wherein from 2 to 10 amino acid residues) are deleted from the amino (preferably from 2 to 30 amino acid residues, more preferably acid sequence of SEQ ID NO: 34 are substituted preferably from 2 to 10 amino acid residues) in the amino residues (preferably from 2 to 30 amino acid residues, more 34, and amino acid sequences wherein one or more amino acid residues) are added to the amino acid sequence of SEQ ID NO:

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(ii) an amino acid sequence selected from the group consisting with one or more other amino acid residues, or/and of an amino acid sequence represented by SEQ ID NO: 35, residues (preferably from 2 to 30 amino acid residues, more amino acid sequences wherein one or more amino acid

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preferably from 2 to 10 amino acid residues) are deleted

with one or more other amino acid residues, of SEQ ID NO: 35, and amino acid sequences wherein one or sequences wherein one or more amino acid residues (preferably from the amino acid sequence of SEQ ID NO: 35, amino acid more amino acid residues (preferably from 2 to 30 amino acid 10 amino acid residues) are added to the amino acid sequence from 2 to 30 amino acid residues, more preferably from 2 to in the amino acid sequence of SEQ ID NO: 35 are substituted residues, more preferably from 2 to 10 amino acid residues)

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#### or a salt thereof;

- 15 20 25 by SEQ ID NO: 38, amino acid sequences wherein one or more from the group consisting of an amino acid sequence represented (preferably from 2 to 30 amino acid residues, more preferably amino acid sequences wherein one or more amino acid residues are deleted from the amino acid sequence of SEQ ID NO: 38, residues, more preferably from 2 to 10 amino acid residues) amino acid residues (preferably from 2 to 30 amino acid to the above (38) comprising an amino acid sequence selected the amino acid sequence of SEQ ID NO: 38 are substituted with or more amino acid residues (preferably from 2 to 30 amino acid sequence of SEQ ID NO: 38, and amino acid sequences wherein one from 2 to 10 amino acid residues) are added to the amino acid one or more other amino acid residues, or a salt thereof; residues, more preferably from 2 to 10 amino acid residues) in (70) a G protein coupled receptor protein according
- <u>۵</u> 35 by SEQ ID NO: 39, amino acid sequences wherein one or more to the above (39) comprising an amino acid sequence selected amino acid sequences wherein one or more amino acid residues are deleted from the amino acid sequence of SEQ ID NO: 39, residues, more preferably from 2 to 10 amino acid residues) amino acid residues (preferably from 2 to 30 amino acid from the group consisting of an amino acid sequence represented sequence of SEQ ID NO: 39, and amino acid sequences wherein one from 2 to 10 amino acid residues) are added to the amino acid (preferably from 2 to 30 amino acid residues, more preferably (71) a G protein coupled receptor protein according

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or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 39 are substituted with one or more other amino acid residues, or a salt thereof;

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to the above (40) comprising an amino acid sequence selected from the group consisting of an amino acid sequence selected by SEQ ID NO: 56, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 56, amino acid sequence of SEQ ID NO: 56, amino acid sequences wherein one or more amino acid sequences wherein one or more amino acid residues) from 2 to 10 amino acid residues, more preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 56, and amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues) in the amino acid sequence of SEQ ID NO: 56 are substituted with one or more other amino acid residues, or a salt thereof;

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the above (61) wherein said ligand is selected from the group consisting of angiotensin, bombesin, canavinoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, VIP (vasoactive intestinal and related peptides), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene related peptides), adrenomedullin, leukotriene, pancreastatin, prostaglandin, thromboxanes, adenosine, adrenaline, a - and B - chemokine (IL-8, GROa , GROB , GROT , NAP-2, ENA-78, PP4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1a, MIP-1B , RANTES, etc.), endothelin, enterogastrin, histamine, neurotensin, TRH, pancreatic polypeptides and galanin;

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salt thereof capable of inhibiting the binding of a ligand with a G protein coupled receptor protein according to any of the above (33) to (40), which comprises measuring amounts of a labeled ligand bound to the said G protein coupled receptor

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protein in at least two cases:

- (i) where the labeled ligand is contacted with at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof, and
  - (ii) where the labeled ligand together with a compound to be tested is contacted with at least one component elected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof.

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- 15 and comparing the measured amounts of the labeled ligand;
- salt thereof capable of inhibiting the binding of a ligand with a G protein coupled receptor protein according to any of the above (33) to (40), which comprises measuring amounts of a labeled ligand bound to a cell comprising the said G protein coupled receptor protein in at least two cases:

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- (i) where the labeled ligand is contacted with the said cell, and
- (ii) where the labeled ligand together with a compound to be tested is contacted with the said cell, and comparing the measured amounts of the labeled ligand;

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salt thereof capable of inhibiting the binding of a ligand with a G protein coupled receptor protein according to any of the above (33) to (40), which comprises measuring amounts of a labeled ligand bound to a membrane fraction of a cell comprising the said G protein coupled receptor protein in at least two cases:

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(i) where the labeled ligand is contacted with the said membrane fraction, and

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(ii) where the labeled ligand together with a compound to be tested is contacted with the membrane fraction,

and comparing the measured amounts of the labeled ligand

- above (33) to (40), which comprises measuring amounts of a a G protein coupled receptor protein according to any of the in at least two cases: labeled ligand bound to said G protein coupled receptor protein salt thereof capable of inhibiting the binding of a ligand with (77) a method for the screening of a compound or a
- (i) where the labeled ligand is contacted with a G protein coupled receptor protein according to any of the above incubation of the transformant, and transformant according to the above (59) during (33) to (40) which is expressed on the membrane of a

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(ii) where the labeled ligand together with a compound to incubation of the transformant, transformant according to the above (59) during receptor protein according to any of the above be tested is contacted with the G protein coupled (33) to (40) which is expressed on the membrane of a

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and comparing the measured amounts of the labeled ligand

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- a G protein coupled receptor protein according to any of the salt thereof capable of inhibiting the binding of a ligand with above (33) to (40), which comprises measuring G protein coupled receptor protein-mediated cell-stimulating activities in at (78) a method for the screening of a compound or a
- (i) where a compound capable of activating the G protein said G protein coupled receptor protein, and coupled receptor protein according to any of the above (33) to (40) is contacted with a cell comprising the
- (ii) where the compound capable of activating the G protein together with a compound to be tested is contacted with receptor protein, the cell comprising the said G protein coupled

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and comparing the measured cell-stimulating activities;

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a G protein coupled receptor protein according to any of the salt thereof capable of inhibiting the binding of a ligand with a method for the screening of a compound or a

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least two cases: receptor protein-mediated cell-stimulating activities in at above (33) to (40), which comprises measuring G protein coupled

(i) where a compound capable of activating the G protein transformant according to the above (59) during receptor protein according to any of the above (33) to (40) is contacted with a G protein coupled coupled receptor protein according to any of the above incubation of the transformant, and (33) to (40) which is expressed on the membrane of a

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(ii) where the compound capable of activating the G expressed on the membrane of a transformant according protein together with a compound to be tested is transformant, to the above (59) during incubation of the according to any of the above (33) to (40) which is contacted with the G protein coupled receptor protein

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and comparing the measured cell-stimulating activities;

- 20 melatonin, neuropeptide Y, opioid, purine, vasopressin, bombesin, canavinoid, cholecystokinin, glutamine, serotonin, coupled receptor protein according to any of the above (33) to (40) is selected from the group consisting of angiotensin, wherein said compound capable of activating the G protein (80) a method according to the above (78) or (79)
- 30 25 oxytocin, VIP (vasoactive intestinal and related peptides), MIP1 $\alpha$  , MIP-1 $\beta$  , RANTES, etc.), endothelin, enterogastrin, NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, adrenaline, a - and  $\beta$  -chemokine (IL-8, GRO $\alpha$ , GRO $\beta$ , GRO $\gamma$ , pancreastatin, prostaglandin, thromboxane, adenosine somatostatin, dopamine, motilin, amylin, bradykinin, CGRP histamine, neurotensin, TRH, pancreatic polypeptides and (calcitonin gene related peptides), adrenomedullin, leukotriene,
- ω 5 according to any of the above (62) and (74) to (80) or a salt a compound which is determined through a method
- a pharmaceutical composition comprising an

- 53 -

effective amount of a compound according to the above (81) or a salt thereof;

(83) a screening kit according to the above (63), comprising a cell comprising a G protein coupled receptor protein according to any of the above (33) to (40);

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- (84) a screening kit according to the above (63), comprising a membrane fraction derived from a cell comprising a G protein coupled receptor protein according to any of the above (33) to (40);
- (85) a screening kit according to the above (63), comprising a cell of the (59) or (109) mentioned herein below;

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- (86) a screening kit according to the above (63), comprising a membrane fraction derived from a cell of the (59) or (109);
- (87) a compound which is determined by means of a screening kit according to any of the above (63) and (83) to (86) or a salt thereof;

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(88) a pharmaceutical composition comprising an effective amount of a compound according to the above (87) or a salt thereof; and

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selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof, which comprises contacting an antibody according to the above (64) with the component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide segments or salts thereof according to the above (41), and mixtures thereof.

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Still another aspect of the present invention is

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(90) a ligand to a G protein coupled receptor protein according to any of the above (33) to (40), which is determined through the following step of: contacting (i) at least one component selected from the group consisting of G protein coupled receptor proteins

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or salts thereof according to any of the above

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(33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof,

with (ii) at least one compound to be examined; and

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- (91) a compound capable of inhibiting the binding of a G protein coupled receptor protein according to any of the above (33) to (40) with a ligand, which is determined through carrying out a comparison between:
- (i) at least one case where said ligand is contacted with at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof

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according to the above (41), and mixtures thereof, and

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(ii) at least one case where said ligand together with a compound to be tested is contacted with at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the

Another aspect of the present invention is

above (41), and mixtures thereof.

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(92) a recombinant G protein coupled receptor protein and a salt thereof which is obtained by the expression of a DNA according to any of the above (42) to (57), or a modified or fragmented derivative thereof;

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(93) a method for amplifying a DNA coding for G protein coupled receptor protein by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

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- a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template, and
- (2) at least one DNA primer selected from the group consisting of DNA primers comprising either SEQ ID NO: 1 or SEQ ID NO: 2; and

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(94) a method for screening DNA libraries for

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a DNA coding for G protein coupled receptor protein, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

(1) said DNA library, and

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(2) at least one DNA primer selected from the group consisting of DNA primers comprising either SEQ ID NO: 1 or SEQ ID NO: 2,

to amplify selectively the DNA coding for G protein coupled receptor protein, contained in the DNA library.

Yet another aspect of the present invention is

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(95) a monoclonal antibody against at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof;

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(96) a preparation of purified polyclonal antibodies against at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof;

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- (97) an immunoassay for detecting a G protein coupled receptor protein which comprising
- (i) incubating a sample to be tested with an antibody according to the above (64) to allow formation of an antigenantibody complex; and
- (ii) detecting an antigen-antibody complex formed in step (i); and
- (98) an immunoassay for detecting antibodies against a G protein coupled receptor protein which comprising

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(i) incubating a sample to be tested with at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof to allow formation of an antigen-antibody complex; and

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(ii) detecting an antigen-antibody complex formed in step (a).

Still another aspect of the present invention is (99) an antisense DNA or RNA which comprises a

- 5 nucleotide sequence complementary to at least a portion of a DNA according to any of the above (42) to (57), said antisense DNA or RNA being hybridizable to said DNA according to any of the above (42) to (57);
- (100) an antisense DNA or RNA according to the above
  (99) wherein said antisense DNA or RNA comprises the 5' end
  hairpin loop, 5' end 6-base-pair repeat, 5' end untranslated
  region, protein translation initiation site or codon, ORF
  translation initiation site or codon, 3'-untranslated region,
  3' end palindrome region, or 3' end hairpin loop of a G protein
  coupled receptor protein DNA according to any of the above
  (42) to (57);
- (101) an antisense DNA or RNA according to the above
   (99) in a pharmaceutically acceptable carrier;
- (102) an antisense DNA or RNA according to the above (99) comprising from 2 to 50 nucleotides;

- (103) a method for modulating the activity of a G protein coupled receptor protein comprising contacting cells expressing the G protein coupled receptor protein with an antisense DNA or RNA according to the above (99);
- protein coupled receptor protein according to any of the above (33) to (40), which comprises administering to an individual at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof; and
- (105) a method for producing a hybridoma which produces a monoclonal antibody against a G protein coupled receptor protein according to any of the above (33) to (40), which comprises
- (i) immunizing an individual with at least one

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the above (33) to (40), peptide fragments or segments or salts coupled receptor proteins or salts thereof according to any of thereof according to the above (41), and mixtures thereof; component selected from the group consisting of G protein

- (ii) immortalizing antibody producing cells from the immunized individual;
- (iii) selecting an immortal cell which produces antibodies reactive with the G protein coupled receptor protein; and
- (iv) growing said immortal cell.

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sequence) coding for G protein coupled receptor protein in a (106) a PCR screening kit for a DNA (or nucleotide Yet another aspect of the present invention is

DNA library which comprises

consisting of DNA primers comprising a nucleotide at least one DNA primer selected from the group (i) (H) 72

represented by SEQ ID NO: 16 and DNA primers comprising primers comprising a nucleotide sequence represented by a nucleotide seguence represented by SEQ ID NO: 18, and comprising a nucleotide sequence represented by SEQ ID comprising a nucleotide sequence represented by SEQ ID represented by SEQ ID NO: 5, DNA primers comprising a NO: 14, DNA primers comprising a nucleotide sequence nucleotide sequence represented by SEQ ID NO: 6, DNA NO: 3, DNA primers comprising a nucleotide sequence sequence represented by SEQ ID NO: 10, DNA primers SEQ ID NO: 7, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1, DNA primers

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primers comprising a nucleotide sequence represented by comprising a nucleotide sequence represented by SEQ ID represented by SEQ ID NO: 8, DNA primers comprising a nucleotide seguence represented by SEQ ID NO: 9, DNA NO: 4, DNA primers comprising a nucleotide sequence SEQ ID NO: 11, DNA primers comprising a nucleotide consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers at least one DNA primer selected from the group 0

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SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by sequence represented by SEQ ID NO: 19; or

- sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ consisting of DNA primers comprising a nucleotide at least one DNA primer selected from the group NO: 12, and (ii)
- consisting of DNA primers comprising a nucleotide at least one DNA primer selected from the group sequence represented by SEQ ID NO: 13; 0

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(107) a vector comprising the DNA according to the

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- protein DNA according to any of the above (7) and (42) to (57), (108) an expression system comprising an open reading frame (ORF) of DNA derived from a G protein coupled receptor wherein the ORF is operably linked to a control sequence compatible with a desired host cell;
- carrying a vector of the above (107) or an expression system of (109) a transformant (including a transfectant) the above (108);

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coupled receptor protein on the membrane of the transformant; the transformant of the above (109) to express said G protein receptor protein or a salt thereof, which comprises culturing (110) a process for producing a G protein coupled

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- (111) a method for expressing a polypeptide of G protein coupled receptor protein, comprising:
- (a) providing a transformant of the above (59) or (109); and

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- (b) incubating the transformant under conditions which allow expression of the polypeptide of G protein coupled receptor protein;
- (112) a method for preparing a transformant according to the above (59) or (109), comprising:

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(a) providing a host cell capable of transformation;

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or (107) or an expression system according to the above (108); (b) providing a vector according to the above (58)

allow transformation of the host cell with the vector or the expression system; (c) incubating (a) with (b) under conditions which

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(113) a pharmaceutical composition according to the

pharmaceutically acceptable carrier, excipient or diluent; pharmaceutically acceptable salt thereof in admixture with a compound according to the above (81) or (87) or a above (82) or (88), comprising an effective amount of a

above (82) or (88), for inhibiting the binding of a G protein coupled receptor protein according to the present invention (114) the pharmaceutical composition according to the

with a ligand; (115) a method for inhibiting the binding of a G

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the above (81) or (87) or a salt thereof with said medium; protein coupled receptor protein comprising contacting cells contacting an effective amount of a compound according to invention with a ligand in a medium which comprises protein coupled receptor protein according to the present an effective amount of a compound according to the above (81) expressing the G protein coupled receptor protein with a (116) a method for modulating the activity of a G

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or (87) or a salt thereof; (117) the ligand according to the above (90) being

labeled with a detectable reporter;

wherein the antibody is labeled with a detectable reporter; (118) the antibody according to the above (64)

an expression of G protein coupled receptor protein, which comprises an effective amount of the antisense DNA according to the above (99), and (119) a pharmaceutical composition for controlling

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according to the above (59) or (109). (120) a culture product produced by a transformant

(121) a DNA according to the above (1) wherein the Yet another aspect of the present invention is

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DNA is an oligonuclectide having from 8 to 60 base residues; (122) a DNA according to the above (1) wherein the

DNA is synthetic;

S protein coupled receptor protein or a fragment thereof, which is obtained through the method according to any of the above (123) a DNA (or nucleotide sequence) coding for a G

(5) to (32); (124) a DNA (or nucleotide sequence) according to

10 15 20 protein is selected from the group consisting of angiotensin cholecystokinin receptor, glutamine receptor, serotonin receptor, bombesin receptor, canavinoid receptor, the above (123), wherein said G protein coupled receptor receptor, VIP receptor (vasoactive intestinal and related receptor, purine receptor, vasopressin receptor, oxytocin receptor, melatonin receptor, neuropeptide y receptor, opioid motilin receptor, amylin receptor, bradykinin receptor, CGRP peptide receptor), somatostatin receptor, dopamine receptor, receptor, prostaglandin receptor, thromboxane receptor, adrenomedullin receptor, leukotriene receptor, pancreastatin receptor (calcitonin gene related peptide receptor), receptor including IL-8, GROlpha , GROeta , GROeta , NAP-2, ENA-78, adenosine receptor, adrenaline receptor, a - and  $oldsymbol{eta}$  -chemokine receptor; and receptor, pancreatic polypeptide receptor, and galanin receptor, histamine receptor, neurotensin receptor, TRH and RANTES receptors, endothelin receptor, enterogastrin PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1a , MIP-1 $\beta$  , (125) a culture product produced by a transformant

35 substantially the same. Substitutions, deletions or ligand binding activity, and physical characteristics are means that the activity of the protein, e.g., nature of the in which case polypeptides containing the substitution, in the physical and chemical characteristics of a polypeptide, insertions of amino acids often do not produce radical changes As used herein the term "substantial equivalent(s)"

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according to the above (59) or (109).

leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, deletion, or insertion would be considered to be substantially equivalent to polypeptides lacking the substitution, deletion, glutamine, The positively charged (basic) amino acids include (acidic) amino acids include aspartic acid and glutamic acid. or insertion. Substantially equivalent substitutes for an amino acid within the sequence may be selected from other The non-polar (hydrophobic) amino acids include alanine, arginine, lysine and histidine. The negatively charged serine, threonine, cysteine, tyrosine, asparagine, and members of the class to which the amino acid belongs.

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## BRIEF DESCRIPTION OF THE DRAWINGS

nucleotide sequence each of other G protein coupled receptor Figure 1 depicts the community (homology) of the sequence of 5' side synthetic DNA primers (HS-1) having a nucleotide sequence represented by SEQ ID NO: 1 with the Protein-encoding cDNAs and genes.

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Primers (HS-2) having a nucleotide sequence represented by SEQ ID NO: 2 with the nucleotide sequence each of other G protein Figure 2 depicts the community (homology) of the sequence which is complementary to 3' side synthetic DNA coupled receptor protein-encoding cDNAs and genes.

represented by SEQ ID NO: 6 relative to the nucleotide seguence each of other G protein coupled receptor protein-encoding cDNAs Figure 3 depicts the community (homology) of the nucleotide sequence represented by SEQ ID NO: 5 or 5' side sequence of 5' side synthetic DNA primers (3A) having a synthetic DNA primers (3B) having a nucleotide sequence and genes.

represented by SEQ ID NO: 3 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs Figure 4 depicts the community (homology) of the nucleotide seguence represented by SEQ ID NO: 7 or 5' side sequence of 5' side synthetic DNA primers (3C) having a synthetic DNA primers (3D) having a nucleotide seguence

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NO: 8 or the nucleotide sequence (6B) which is complementary to represented by SEQ ID NO: 9 relative to the nucleotide seguence sequence (6A) which is complementary to 3' side synthetic DNA each of other G protein coupled receptor protein-encoding cDM Figure 5 depicts the community (homology) of the Primers having a nucleotide sequence represented by SEQ ID 3' side synthetic DNA primers having a nucleotide sequence

sequence (6C) which is complementary to 3' side synthetic DNA Primers having a nucleotide sequence represented by SEQ ID Figure 6 depicts the community (homology) of the protein coupled receptor protein-encoding cDNAs and genes. NO: 4 relative to the nucleotide sequence each of other G

nucleotide sequence each of other G protein coupled receptor Figure 7 depicts the community (homology) of the nucleotide sequence represented by SEQ ID NO: 10 with the sequence of 5' side synthetic DNA primers (T2A) having a Protein-encoding cDNAs and genes.

ID NO: 11 relative to the nucleotide sequence each of other G Primers (T7A) having a nucleotide sequence represented by SEQ protein coupled receptor protein-encoding cDNAs and genes. Figure 8 depicts the community (homology) of the sequence which is complementary to 3' side synthetic DNA

sequence of 5' side synthetic DNA primers (TM1-A2) having a 🕌 nucleotide sequence represented by SEQ ID NO: 12 relative to Figure 9 depicts the community (homology) of the the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

SEQ ID NO: 13 relative to the nucleotide seguence each of other Primers (TM3-B2) having a nucleotide sequence represented by G protein coupled receptor protein-encoding cDNAs and genes. Figure 10 depicts the community (homology) of the sequence which is complementary to 3' side synthetic DNA

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nucleotide sequence represented by SEQ ID NO: 14 relative to sequence of 5' side synthetic DNA primers (TM3-C2) having a Figure 11 depicts the community (homology) of the

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receptor protein-encoding cDNAs and genes. the nucleotide sequence each of other G protein coupled

G protein coupled receptor protein-encoding cDNAs and genes. SEQ ID NO: 15 relative to the nucleotide sequence each of other primers (TM6-E2) having a nucleotide sequence represented by sequence which is complementary to 3' side synthetic DNA Figure 12 depicts the community (homology) of the

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receptor protein-encoding cDNAs and genes. the nucleotide sequence each of other G protein coupled nucleotide sequence represented by SEQ ID NO: 16 relative to sequence of 5' side synthetic DNA primers (TM2F18) having a Figure 13 depicts the community (homology) of the

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G protein coupled receptor protein-encoding cDNAs and genes. primers (TM6R21) having a nucleotide sequence represented by sequence which is complementary to 3' side synthetic DNA SEQ ID NO: 17 relative to the nucleotide sequence each of other Figure 15 depicts the community (homology) of the Figure 14 depicts the community (homology) of the

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nucleotide sequence represented by SEQ ID NO: 18 relative to receptor protein-encoding cDNAs and genes. the nucleotide sequence each of other G protein coupled sequence of 5' side synthetic DNA primers (S3A) having a

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primers (S6A) having a nucleotide sequence represented by SEQ protein coupled receptor protein-encoding cDNAs and genes. ID NO: 19 relative to the nucleotide sequence each of other G sequence which is complementary to 3' side synthetic DNA Figure 16 depicts the community (homology) of the

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S primers having a nucleotide sequence represented by SEQ ID NO: amygdala (1, 2, 7), human pituitary body (3, 4, 8) and rat profile of cDNA products each obtained from human brain PCR is carried out under mild conditions, and M denotes a size disclosed in Examples, lanes 7 to 9 show the results of when results of when PCR is carried out under severe conditions as represented by SEQ ID NO: 2, wherein lanes 1 to 6 show the brain (5, 6, 9) by PCR amplification using the synthetic DNA and the synthetic DNA primers having a nucleotide sequence Figure 17 is the 1.2% agarose gel electrophoresis

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restriction enzyme, EcoT14I. marker which is obtained by cutting  $\lambda$  -phage DNA with

clone A58 is obtained by amplifying human brain amygdalasequencing of clone A58 with a T7 primer wherein the Figure 18 shows the nucleotide sequence determined by

derived cDNA by PCR under mild conditions and subcloning it to Figure 19 shows the nucleotide sequence determined by

sequencing of clone A58 with an SP6 primer.

to pCR II. clone 57-A-2 is obtained by amplifying human brain amygdaladerived cDNA by PCR under severe conditions and subcloning it sequencing of clone 57-A-2 by using a -21M13 primer wherein the Figure 20 shows the nucleotide sequence determined by

cDNA by PCR under mild conditions and subcloning it to clone B54 is obtained by amplifying rat whole brain-derived sequencing of clone B54 with a T7 primer wherein the Figure 21 shows the nucleotide sequence determined by

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for sequencing is -21M13, and the underlined part corresponds amino acid sequence encoded thereby, wherein the primer used by PCR using a human pituitary gland-derived cDNA and the protein cDNA fragment included in the cDNA clone p1972 isolated human pituitary gland-derived G protein coupled receptor to the synthetic primer. Figure 22 illustrates the nucleotide sequence of the

by PCR using a human pituitary gland-derived cDNA and the protein cDNA fragment included in the cDNA clone p19P2 isolated human pituitary gland-derived G protein coupled receptor part corresponds to the synthetic primer. amino acid sequence encoded thereby, wherein the primer used for sequencing is M13RV-N (Takara, Japan), and the underlined Figure 23 illustrates the nucleotide sequence of the

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included in p19P2, prepared based upon the amino acid sequence derived G protein coupled receptor protein cDNA fragment profile of the protein encoded by the human pituitary gland-Figure 24 is the partial hydrophobicity plotting

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shown in Figure 22.

Figure 25 is the partial hydrophobicity plotting profile of the protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2, prepared based upon the amino acid sequence shown in Pigure 23.

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Figure 26 shows the partial amino acid sequence (p19P2) of the protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2, as shown in Figures 22 and 23, relative to the known G protein coupled receptor protein, S12863, wherein reverse amino acid residues are in agreement, the 1st to 99th amino acid residues in Figure 22, and the 156th to 230th amino acid residues thereof correspond to the amino acid residues thereof correspond to the 1st to 68th amino acid residues in Figure 23.

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Figure 27 is the nucleotide sequence of the MIN6-derived G protein coupled receptor protein cDNA fragment derived based upon the nucleotide sequences of the MIN6-derived G protein coupled receptor protein cDNA fragments each included in the cDNA clones, pG3-2 and pG1-10, isolated by PCR using a MIN6-derived cDNA and the amino acid sequence encoded thereby, wherein the underlined parts corresponds to the synthetic primers.

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Figure 28 is the partial hydrophobicity plotting profile of the MIN6-derived G protein coupled receptor protein, prepared based upon the partial amino acid sequence shown in Figure 27.

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Figure 29 is the partial nucleotide sequence of the novel receptor protein cDNA clone, p63A2, obtained from the human amygdaloid nucleus by PCR amplification and the amino acid sequence encoded thereby, wherein the underlined part corresponds to the synthetic primer.

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Figure 30 is the partial nucleotide sequence of the novel receptor protein cDNA clone, p63A2, obtained from the human amygdaloid nucleus by PCR amplification and the amino acid sequence encoded thereby, wherein the underlined part

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corresponds to the synthetic primer.

Figure 31 is the hydrophobicity plotting profile, prepared based upon the amino acid sequence shown in Figure 29, suggesting the presence of hydrophobic domains as designated by 1 to 3.

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Figure 32 is the hydrophobicity plotting profile, prepared based upon the amino acid sequence shown in Figure 30, suggesting the presence of hydrophobic domains as designated by 4 to 6.

Figure 33 is the partial amino acid sequence (p63A2) of the protein encoded by the novel receptor protein cDNA fragment included in p63A2, relative to the partial amino acid sequence of the G protein coupled receptor protein (P30731) expressed and induced by a mouse T cell-derived glucocorticoid, wherein reverse amino acid residues are in agreement.

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Figure 34 is the whole nucleotide sequence of the the human pituitary gland-derived G protein coupled receptor protein cDNA, included in the cDNA clone, phGR3, isolated from the human-derived cDNA library by plaque hybridization using an DNA insert in the p19P2 as a probe, and the amino acid sequence encoded thereby.

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Figure 35 is the northern blotting profile of the human pituitary gland mRNA of the receptor gene encoded by the human pituitary gland-derived cDNA clone, phGR3.

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Figure 36 is the hydrophobicity plotting profile the protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA included in phGR3, prepared based upon the amino acid sequence shown in Figure 34.

Figure 37 is the partial nucleotide sequence of the novel receptor protein cDNA clone, p3H2-17, obtained from mouse pancreatic  $\beta$ -cell strain, MIN6, by PCR amplification and the amino acid sequence encoded thereby, wherein the underlined part corresponds to the synthetic primer used for the PCR amplification.

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Figure 38 is the hydrophobicity plotting profile, prepared based upon the amino acid sequence shown in Figure 37, suggesting the presence of hydrophobic domains as designated

protein (JN0605) and bovine neuropeptide Y receptor protein 3 protein (A46226), human somatostatin receptor subtype 4 receptor protein (P34996), human somatostatin receptor subtype by 3 to 6. relative to the partial amino acid sequence each of chicken ATP by the novel receptor protein cDNA included in p3H2-17, (S28787), wherein reverse amino acid residues are in agreement. Figure 39 is the partial amino acid sequence encoded

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underlined parts correspond to the synthetic primers used for and the amino acid sequence encoded thereby, wherein the mouse pancreatic eta -cell strain, MIN6, by PCR amplification novel receptor protein cDNA clone, p3H2-34, obtained from the PCR amplification. Figure 40 is the partial nucleotide sequence of the

hydrophobicity, the axis of abscissa represents the number of wherein the axis of ordinate represents an index of prepared based upon the amino acid sequence shown in Figure 40, hydrophobic domains. amino acids and numerals 3 to 6 represent the presence of Figure 41 is the hydrophobicity plotting profile,

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derived ligand unknown receptor protein (A39297), wherein somatostatin receptor subtype 2 protein (B41795) and ratsomatostatin receptor subtype 4 protein (JN0605), human by the novel receptor protein cDNA included in p3H2-34, reverse amino acid residues are in agreement. relative to the partial amino acid sequence each of human Figure 42 is the partial amino acid seguence encoded

sequence encoded thereby, wherein the underlined parts part smooth muscles by PCR amplification, and the amino acid protein cDNA clone, pMD4, obtained from rabbit gastropyrolic receptor protein cDNA fragment included in the novel receptor gastropyrolic part smooth muscle-derived G protein coupled correspond to the synthetic primers used for the PCR amplification. Figure 43 is the nucleotide sequence of the rabbit

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the protein encoded by the rabbit gastropyrolic part smooth Figure 44 is the hydrophobicity plotting profile of ű

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presence of hydrophobic domains. shown in Figure 35, wherein numerals 1 to 3 suggest the included in pMD4, prepared based upon the amino acid sequence muscle-derived G protein coupled receptor protein cDNA fragment

10 protein (A35639), wherein reverse amino acid residues are in protein coupled receptor protein, rat ligand unknown receptor muscle-derived G protein coupled receptor protein cDNA fragment of the protein encoded by the rabbit gastropyrolic part smooth sequence correspond to the 1st to 88th amino acid residues in agreement, the 1st to 88th amino acid residues of the pMD4 included in pMD4 as shown in Figure 43, relative to the known G Figure 43. Figure 45 is the partial amino acid sequence (pMD4)

15 derived galanin receptor protein cDNA clone, pMGR20, which has the amino acid sequence encoded thereby. been cloned with, as a probe, the cDNA insert in p3H2-34 and Figure 46 shows the nucleotide sequence of the mouse-

prepared based upon the amino acid sequence shown in Figure 46, wherein the axis of ordinate represents an index of hydrophobic property, the axis of abscissa represents the presence of hydrophobic domains. number of amino acids, and numerals 1 to 7 represent the Figure 47 is the hydrophobicity plotting profile,

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derived galanin receptor protein, wherein reverse amino acid relative to the amino acid sequence (HUMAGALAMI) of the humanof the mouse-derived galanin receptor protein encoded by pMGR20, residues are in agreement. Figure 48 is the amino acid sequence (MOUSEGALRECE)

protein cDNA clone, pMJ10, obtained from rabbit gastropyrolic gastropyrolic part smooth muscle-derived G protein coupled corresponds to the synthetic primers used for the PCR sequence encoded thereby, wherein the underlined parts part smooth muscles by PCR amplification and the amino acid receptor protein cDNA fragment included in the novel receptor amplification. Figure 49 is the nucleotide sequence of the rabbit

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Figure 50 is the hydrophobicity plotting profile of

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the protein encoded by the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDBA fragment included in pMJ10, prepared based upon the amino acid sequence shown in Figure 49, wherein numerals 4 to 6 suggest the presence of hydrophobic domains.

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Figure 51 is the partial amino acid sequence (pwJ10) of the protein encoded by the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in pMJ10 shown in Figure 49, relative to human ligand unknown receptor protein (B42009), human N-formylpeptide receptor protein (JC2014), rabbit N-formylpeptide receptor protein (A46520), mouse C5a anaphylatoxin receptor protein (A46525) and bovine neuropeptide Y receptor protein (S28787) which are known G protein coupled receptor proteins, wherein reverse amino acid residues are in agreement, and the 1st to 125th amino acid residues of pMJ10 correspond to the 1st to

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Figure 52 is the nucleotide sequence of the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in the novel receptor protein cDNA clone, pMH28, obtained from rabbit gastropyrolic part smooth muscles by PCR amplification and the amino acid sequence encoded thereby, wherein the underlined parts correspond to the synthetic primers used for the PCR amplification.

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Figure 53 is the hydrophobicity plotting profile of the protein encoded by the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDBA fragment included in pMH28, prepared based upon the amino acid sequence shown in Figure 52, wherein numerals 4 to 6 suggest the presence of hydrophobic domains.

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Figure 54 is the partial amino acid sequence (pMH28) of the protein encoded by the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in pMH28 shown in Figure 52, relative to mouse IL-8 receptor protein (P35343), human somatostatin receptor protein 1 (A41795) and human somatostatin receptor protein

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which are known G protein coupled receptor proteins, wherein reverse amino acid residues are in agreement, and the 1st to 119th amino acid residues of pMH28 correspond to the 1st to 119th amino acid residues in Figure 52.

Figure 55 is the nucleotide sequence of the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in the novel receptor protein cDNA clone, pMN7, obtained from rabbit gastropyrolic part smooth muscles by PCR amplification and the amino acid sequence encoded thereby, wherein the underlined 5'-end nucleotide sequence part corresponds to the synthetic primer used for the PCR amplification.

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Figure 56 is the nucleotide sequence of the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in the novel receptor protein cDNA clone, pMN7, obtained from rabbit gastropyrolic part smooth muscles by PCR amplification and the amino acid sequence encoded thereby, wherein the underlined 3'-end nucleotide sequence part corresponds to the synthetic primer used for the PCR amplification.

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the protein encoded by the rabbit gastropyrolic part smooth muscle- derived G protein coupled receptor protein cDNA fragment included in pMN7, prepared based upon the amino acid sequenceshown in Figures 55 and 56, wherein numerals TM2 to TM6 suggithe presence of hydrophobic domains.

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Figure 58 is the partial hydrophobicity plotting profile of the protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2, prepared based upon the amino acid sequence shown in Figure 22.

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Figure 59 is the partial hydrophobicity plotting profile of the protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2, prepared based upon the amino acid sequence shown in Figure 23.

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Figure 60 shows the partial amino acid seguence

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reverse amino acid residues are in agreement, the 1st to 99th derived G protein coupled receptor protein cDNA fragment amino acid residues of the p19P2 sequence correspond to the 1st the known G protein coupled receptor protein, \$12863, wherein included in p19p2, as shown in Figures 22 and 23, relative to amino acid residues in Figure 23. 230th amino acid residues thereof correspond to the 1st to 68th to 99th amino acid residues in Figure 22, and the 156th to (p19p2) of the protein encoded by the human pituitary gland-

protein, as shown in Figure 27, relative to the partial amino in agreement, the 1st to 99th amino acid residues of the in Figures 22 and 23, wherein reverse amino acid residues are acid seguence (p19P2) of the protein encoded by p19P2, as shown Figure 23, and the 1st to 223rd amino acid residues of the thereof correspond to the 1st to 68th amino acid residues in residues in Figure 22, the 156th to 223rd amino acid residues p19P2 sequence correspond to the 1st to 99th amino acid (pG3-2/pG1-10) of the MIN6-derived G protein coupled receptor pG3-2/pG1-10 sequence correspond to the 1st to 223rd amino acid residues in Figure 27. Figure 61 is the partial amino acid sequence

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wherein the underlined parts corresponds to the synthetic MIN6-derived cDNA and the amino acid sequence encoded thereby, included in the cDNA clone, p5S38, isolated by PCR using a derived G protein coupled receptor protein cDNA fragment Figure 62 is the nucleotide sequence of the MIN6-

shown in Figure 62, relative to the partial amino acid sequence sequence of the cDNA fragment included in pG3-2 and pG1-10, as encoded by the nucleotide sequence derived from the nucleotide amino acid sequence of the G protein coupled receptor protein p19P2, as shown in Figures 22 and 23, as well as the partial of the MIN6-derived G protein coupled receptor protein, as shown in Figure 27, wherein reverse amino acid residues are in (p19P2) of the G protein coupled receptor protein encoded by agreement, the 1st to 144th amino acid residues of the p5S38 Figure 63 is the partial amino acid sequence (p5S38)

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sequence correspond to the 1st to 144th amino acid residues in correspond to the 1st to 68th amino acid residues in Figure 23, Figure 22, the 156th to 223rd amino acid residues thereof sequence correspond to the 1st to 99th amino acid residues in Figure 62, the 1st to 99th amino acid residues of the p19P2 sequence correspond to the 1st to 223rd amino acid residues in and the 1st to 223rd amino acid residues of the pG3-2/pG1-10

profile of the protein encoded by the MIN6-derived G protein prepared based upon the amino acid sequence shown in Figure 62. coupled receptor protein cDNA fragment included in p5S38, Figure 65 shows the northern blot analysis profile Figure 64 is the partial hydrophobicity plotting

cDNA clone, p3H2-17, for mouse cell line, MIN6, Neuro-2a cell pancreatic  $oldsymbol{eta}$  -cell strain MIN6-derived novel receptor protein of the receptor gene encoded by the cDNA included in the mouse position (unit of number: kb). wherein each arrow and number indicates the size marker and mouse brain, thymus, spleen and pancreas poly(A)  $\bar{R}NA$ ,

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of the receptor gene included in p3H2-17 using mouse thymus analysis profile of the PCR products obtained by 5'RACE PCR and spleen poly(A) RNA. Figure 66 shows the agarose gel electrophoresis

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Lane 1 indicates the size marker 6 (Wako Pure

Chemical, Japan).

NO: 22 with Tag polymerase. thymus-derived PCR product obtained by PCR amplification using the primer having SEQ ID NO: 20 and the primer having SEQ ID Lane 2 indicates the internal control which is the

PCR product obtained by Ex Taq polymerase PCR amplification of thymus cDNA prior to addition of anchors. Lane 3 indicates the negative control which is the

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thymus cDNA prior to addition of anchors. PCR product obtained by Tag polymerase PCR amplification of Lane 4 indicates the negative control which is the

of thymus poly(A) RNA with Pfu polymerase. Lane 5 indicates the PCR product obtained by 5'RACE

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Lane 6 indicates the PCR product obtained by 5'RACE of thymus poly(A)  $^{\dagger}$ RNA with Vent polymerase.

Lane 7 indicates the PCR product obtained by 5'RACE of thymus poly(A)  $^{\dagger} \rm RNA$  with Ex Taq polymerase.

Lane 8 indicates the PCR product obtained by 5'RACE of thymus poly(A)  $^{\dagger} \rm RNA$  with Tag polymerase.

S

Lane 9 indicates the size marker 5 (Wako Pure Chemical, Japan).

Lane 10 indicates the internal control which is the spleen-derived PCR product obtained by PCR amplification using the primer having SEQ ID NO: 20 and the primer having SEQ ID NO: 22 with Tag polymerase.

Lane 11 indicates the negative control which is the PCR product obtained by Ex Tag polymerase PCR amplification of spleen cDNA prior to addition of anchors.

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Lane 12 indicates the negative control which is the PCR product obtained by Tag polymerase PCR amplification of spleen cDNA prior to addition of anchors.

Lane 13 indicates the PCR product obtained by 5'RACE of poly(A) RNA with Pfu polymerase.

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Lane 14 indicates the PCR product obtained by 5'RACE of spleen poly(A) RNA with Vent polymerase.

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Lane 15 indicates the PCR product obtained by 5'RACE of spleen poly(A)  $^{\dagger}$  RNA with Ex Tag polymerase.

Lane 16 indicates the PCR product obtained by 5'RACE of spleen poly(A)  $^{\dagger}$  RNA with Tag polymerase.

22

Lane 17 indicates the size marker 5 (Wako Pure Chemical, Japan).

Each blacked triangle indicates the band recovered.

Figure 67 shows the agarose gel electrophoresis
analysis profile of the PCR products obtained by 3'RACE PCR
of the receptor gene included in p3H2-17 using mouse thymus
and spleen poly(A) \*NNA.

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Lane 1 indicates the size marker 5 (Wako Pure Chemical, Japan).

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Lane 2 indicates the PCR product obtained by 3'RACE of spleen poly(A)  $^{\star}$  RNA with Tag polymerase.

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Lane 3 indicates the PCR product obtained by 3'RACE of spleen poly(A)  $^{\dagger}$ RNA with Ex Tag polymerase.

Lane 4 indicates the PCR product obtained by 3'RACE of spleen poly(A)  $^{\text{RNA}}$  with Vent polymerase.

Lane 5 indicates the PCR product obtained by 3'RACE of spleen poly(A)  $^{\star}$  RNA with Pfu polymerase.

Lane 6 indicates the PCR product obtained by 3'RACE of thymus poly(A) RNA with Tag polymerase.

Lane 7 indicates the PCR product obtained by 3'RACE of thymus poly(A) RNA with Ex Tag polymerase.

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Lane 8 indicates the PCR product obtained by 3'RACE of thymus poly(A)  $^{\dagger}$  RNA with Vent polymerase.

Lane 9 indicates the PCR product obtained by 3'RACE of thymus poly(A) RNA with Pfu polymerase.

Lane 10 indicates the size marker 6 (Wako Pure Chemical, Japan).

Each blacked triangle indicates the band recovered.

Figure 68 depicts the model of the RACE products of the receptor protein cDNA fragment included in p3H2-17 obtained by 5'RACE and 3'RACE. Open squares represent regions which have already been isolated and included in p3H2-17. Small arrows, ①, ②, ③ and ④, indicate the positions and directions of the primers designed in Working Example 19. The big arrow shows a predicted full-length open reading frame of the receptor protein held by p3H2-17. Numbers at both ends, N26, N64, N75, C2, C13 and C15, indicate clone numbers of the RACE products obtained. Among these RACE products, N26, N64 and N75 inserted into pCR II vector and C2, C13 and C15 are inserted into the Smal site of pDC18. The solid triangle indicates the PCR error position which has been clarified through sequencing.

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Figure 69 is the nucleotide sequence of the open reading frame and neighboring regions thereof of mouse G protein coupled receptor protein cDNA included in the cDNA clone pMAH2-17 obtained from mouse spleen and thymus poly(A) RNA by RACE techniques based on the nucleotide sequence of the CDNA fragment included in p3H2-17 and the amino acid

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sequence encoded thereby.

shown in Figure 69. pMAH2-17, prepared based upon the amino acid sequence the protein encoded by the receptor protein cDNA included in Figure 70 is the hydrophobicity plotting profile of

purinoceptor (P2YR CHICK), wherein reverse amino acid residues proteins, mouse  $P_{20}$  purinoceptor (P2UR MOUSE) and chicken  $P_{2\gamma}$ in Figure 69, relative to the known G protein coupled receptor the protein encoded by the mouse-derived G protein coupled receptor protein cDNA fragment included in pMAH2-17, as shown Figure 71 is the amino acid sequence (75+13CODING) of

muscle-derived G protein coupled receptor protein cDNA fragment primer used for the PCR amplification. obtained from rabbit gastropyrolic part smooth muscles by PCR 540th nucleotides) of the rabbit gastropyrolic part smooth wherein the underlined 5' part corresponds to the synthetic amplification, and the amino acid sequence encoded thereby, included in the novel receptor protein cDNA clone, pMN128, Figure 72 is the nucleotide sequence (from 1st to

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are in agreement.

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by PCR amplification, and the amino acid sequence encoded PMN128, obtained from rabbit gastropyrolic part smooth muscles 843rd nucleotides) of the rabbit gastropyrolic part smooth thereby, wherein the underlined 3' part corresponds to the fragment included in the novel receptor protein cDNA clone, muscle-derived G protein coupled receptor protein cDNA synthetic primer used for the PCR amplification. Figure 73 is the nucleotide sequence (from 541st to

the protein encoded by the rabbit gastropyrolic part smooth muscle- derived G protein coupled receptor protein cDNA fragment of hydrophobic domains. sequences shown in Figures 72 and 73, suggesting the presence included in pMN128, prepared based upon the amino acid Figure 74 is the hydrophobicity plotting profile of

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xenopus oocytes injected with cDNA of pMAH2-17-encoded Figure 75 shows inward currents evoked by ATP in

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fragment included in p3H2-17, wherein reverse base residues the mouse-derived G protein coupled receptor protein cDNA included in ph3H2-17, relative to the nucleotide sequence of derived G protein coupled receptor protein cDNA fragment Figure 76 is the nucleotide sequence of the human-

and the amino acid sequence encoded thereby. G protein coupled receptor protein cDNA included in phAH2-17 are in agreement. reading frame and neighboring regions thereof of human-derived Figure 77 is the nucleotide sequence of the open

10 receptor protein cDNA included in phAH2-17. the protein encoded by the human-derived G protein coupled Figure 78 is the hydrophobicity plotting profile of

residues are in agreement. purinoceptor encoded by p3H2-17, wherein reverse amino acid purinoceptor encoded by phAH2-17, relative to the mouse Figure 79 is the amino acid seguence of human type

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

comprising each a nucleotide sequence indicated by a SEQ ID NO a potent primer for polymerase chain reaction in order to NO: 19 have been synthesized and characterized. The DNA is selected from the group consisting of SEQ ID NO: 1 to SEQ ID sequence of G protein coupled receptor protein. PCR amplify DNA sequences encoding part or all of the polypeptide According to the present invention, DNA sequences

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25 ü the polypeptide sequence of G protein coupled receptor protein Screening of DNA libraries for the DNA encoding part or all of be advantageously carried out with the said primer DNA. polypeptide sequence of G protein coupled receptor protein can amplification methods of the DNA coding for part or all of the reaction techniques with the said primer DNA. can be successfully carried out through polymerase chain contained in the DNA library, can be selectively amplified polypeptide sequence of G protein coupled receptor protein, As a result, template DNAs coding for part or all of the and various DNA sequences encoding part or all of the

polypeptide sequence of G protein coupled receptor protein may be isolated and characterized. Further, G protein coupled receptor proteins, peptide segments or fragments derived from the G protein coupled receptor protein, modified derivatives or analogues thereof, and salts thereof may be recognized, predicted, deduced, produced, expressed, isolated and characterized.

The primer DNA useful in PCR amplification of the DNA sequence encoding part or all of the polypeptide sequence of G protein coupled receptor protein is a degenerate deoxynucleotide which has an oligonucleotide sequence to which a SEQ ID NO selected from the group consisting of SEQ ID NO: 19 is assigned.

The nucleotide sequence represented by SEQ ID NO: 1 is a base sequence having the following formula: 5'-CGTGGSCMISSTGGGGAACN, YCCTG-3'

S'-CCTGGSCMTSSTGGGCAACN YCCTG-3' wherein S is G or C, M is A or C, N = A, G, C, or T, and Y is T or C (Figure 1: HS-1).

The nucleotide sequence represented by SEQ ID NO: 2 (HS-2) is a base sequence having the following formula:

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5'-GTN\_GWRRGGCAN\_CCAGCAGARGGCAAA-3' wherein N  $_1$  = A, G, C, or T, W is A or T, R is A or G, and K is G or T, which is complementary to a nucleotide sequence having the following formula:

5'-TTTGCCMTCTGCTGGNTGCCYYWCNAC-3'

wherein N = A, C, G, or T, M is A or C, Y is T or C, and W is A or T (Figure 2).

The nucleotide sequence represented by SEQ ID NO: 3 is a base sequence having the following formula:

5'-ctcgcsgcymtn<sub>2</sub>rgyatggaycgn<sub>2</sub>tat-3'

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wherein S is G or C, Y is C or T, M is A or C, R is A or G, and  $N_2$  = I (Figure 4: 3D). The nucleotide semicanometer  $\frac{1}{2}$ 

The nucleotide sequence represented by SEQ ID NO: 4 is a base sequence having the following formula:

5 '-CATGTRGWAGGGAAN  $_2$  CCAGSAMAN  $_2$  RARRAA-3' wherein R is A or G, W is T or A, S is G or C, M is A or C, and N  $_2$  = I, which is complementary to a nucleotide sequence

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having the following formula:

5'-TTYYTYN\_1TKTSCTGGN\_1TCCCTWCYACATG-3' wherein Y is C or T,  $N_1$  = A, G; C, or T, K is G or T, S is G or C, W is A or T (Figure 6: 6C).

The nucleotide sequence represented by SEQ ID NO: 5 is a base sequence having the following formula:

5'-CTGACYGYTCTN,RSN,RYTGACMGVTAC-3'

wherein Y is C or T, R is A or G, S is G or C, M is A or C, V is A, C or G, and  $N_2$  is I (Figure 3: 3A).

The nucleotide sequence represented by SEQ ID NO; 6 is a base sequence having the following formula:

5'-CTGACYGYTCTN2RSN2RYTGACMGVTAT-3'

wherein Y is C or T, R is A or G, S is G or C, M is A or C, and V is A, C or G, and  $N_2$  is I (Figure 3: 3B).

The nucleotide sequence represented by SEQ ID NO: 7 is a base sequence having the following formula:

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5'-CTCGCSGCYMIN\_RGYATGGAYCGN\_TAC-3'

wherein S is G or C, Y is C or T, M is A or C, R is A or G, and  $N_2$  is I (Figure 4: 3C).

The nucleotide sequence represented by SEQ ID NO: 8 is a base sequence having the following formula: 5'-GATGTGRTARGGSRN,CCAACAGAN,GRYAAA-3'

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wherein R is A or G, S is G or C, Y is C or T, and  $N_2$  is I, which is complementary to a nucleotide sequence having the following formula:

5'-TTTRYCN, TCTGTTGGN, YSCCYTAYCACATC-3'

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wherein R is A or G, Y is C or T, S is G or C, and  $N_1$  is A, T, G, or C (Figure 5: 6A).

The nucleotide sequence represented by SEQ ID NO: 9 is a base sequence having the following formula:

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5'-GATGTGRTARGGSRN, CCAACAGAN, GRYGAA-3'

wherein R is A or G, S is G or C, Y is C or T, and  $N_2$  is I, which is complementary to a nucleotide sequence having the following formula:

35 5'-TTCRYCN<sub>1</sub>TCTGGN<sub>1</sub>YSCCYTAYCACATC-3' wherein R is A or G, Y is C or T, S is G or C, and N<sub>1</sub> is A,

G, or C (Figure 5: 6B).

is a base sequence having the following formula: The nucleotide sequence represented by SEQ ID NO: 10

5'-GYCACCAACN, WSTTCATCCTSWN, HCTG-3'

and  $N_2$  is I (Figure 7: T2A). wherein S is G or C, Y is C or T, W is A or T, H is A, C or T,

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(Figure 8: T7A) is a base sequence having the following The nucleotide sequence represented by SEQ ID NO: 11

5'-ASN<sub>2</sub>SAN<sub>2</sub>RAAGSARTAGAN<sub>2</sub>GAN<sub>2</sub>RGGRTT-3'

complementary to a nucleotide sequence having the following wherein R is A or G, S is G or C, and  $N_2$  is I, which is

5'-AAYCCYN2TCN2TCTAYTSCTTYN2TSN2ST-3'

wherein Y is C or T,  $N_2$  is I, and S is G or C (Figure 8). The nucleotide sequence represented by SEQ ID NO: 12

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is a base sequence having the following formula:

wherein S is G or C, K is G or T, M is A or C, and  $\mathbf{N}_2$  is I (Figure 9: TM1-A2). 5'-TGN2TSSTKMTN2GSN2GTKGTN2GGN2AA-3'

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(Figure 10: TM3-B2) is a base seguence having the following

The nucleotide sequence represented by SEQ ID NO: 13

which is complementary to a nucleotide sequence having the wherein Y is C or T, K is G or T, W is A or T, and  $N_2$  is I, following formula: 5'-AYCKGTAYCKGTCCAN2KGWN2ATKGC-3'

5'-GCMATN<sub>2</sub>WCMN<sub>2</sub>TGGACMGRTACMGRT-3'

wherein M is A or C, W is A or T, R is A or G, and  $\mathrm{N}_2$  is I

30 (Figure 10). The nucleotide sequence represented by SEQ ID NO: 14

wherein K is G or T, S is G or C, Y is C or T, R is A or G, and is a base sequence having the following formula: 5'-CATKKCCSTGGASAGN<sub>2</sub>TAYN<sub>2</sub>TRGC-3'

is I (Figure 11: TM3-C2).

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(Figure 12: TM6-E2) is a base sequence having the following The nucleotide sequence represented by SEQ ID NO: 15

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5'-GWWGGGSAKCCAGCASAN,GGCRAA-3'

 $N_{
m p}$  is I, which is complementary to a nucleotide sequence wherein W is A or T, S is G or C, K is G or T, R is A or G, and

v having the following formula: 5'-TTYGCCN, TSTGCTGGMTSCCCWWC-3'

 $N_2$  is I (Figure 12). wherein Y is C or T, S is G or C, M is A or C, W is A or T, and

is a base sequence having the following formula: The nucleotide sequence represented by SEQ ID NO: 16

5'-ARYYTN2GCN2N2 TN2GCN1GAY-3'

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 $N_2$  is I (Figure 13: TM2F18). wherein R is A or G, Y is C or T, N is A, T, G, or C, and The nucleotide sequence represented by SEQ ID NO: 17

15 (Figure 14: TM6R21) is a base sequence having the following

following formula: which is complementary to a nucleotide sequence having the wherein R is A or G,  ${
m N}_1$  is A, T, G, or C, and  ${
m N}_2$  is I 5'-N2GGN2AN2CCARCAN1AN1N1RN1RAA-3'

(Figure 14).

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is a base sequence having the following formula: The nucleotide sequence represented by SEQ ID NO: 18

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5'-GCCTSN2TN2RN2SATGWSTGTGGAN2MGN2T-3'

wherein S is G or C, R is A or G, W is A or T, M is A or C, and is I (Figure 15: S3A).

(Figure 16: S6A) is a base sequence having the following The nucleotide sequence represented by SEQ ID NO: 19

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5'-GAWSN,TGMYN,AN,RTGGWAGGGN,AN,CCA-3'

R is A or G, and  $\mathrm{N}_2$  is I, which is complementary to a wherein W is A or T, S is G or C, M is A or C, Y is C or T,

nucleotide sequence having the following formula: 5'-TGGN2TN2CCCTWCCAYN2TN2RKCAN2SWTC-3'

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wherein Wishor T, Yis Cor T, Rishor G, Kis Gor T, and S is G or C (Figure 16).

SEQ ID NO: 1 to SEQ ID NO: 19 are degenerate nucleotide primers. indicate the incorporation of plural bases, leading to multiple oligonuclectides in the primer preparation. In other words, aforementioned SEQ ID NOS (R, Y, M, K, S, W, H, V and N) In a specific embodiment, symbols in the

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(transmembrane) domain each of known G protein coupled receptor (Figure 1: HS-1) is a nucleotide sequence highly homologous to somatostatin receptor protein (L14856, HUMSOMATO), rat-derived The nucleotide seguence represented by SEQ ID NO: 1 Proteins such as human-derived TRH receptor protein (HTRHR), human-derived  $C_{\mathsf{c}}$ a receptor protein (HUMC5AAR), human-derived  $\mu$  -Opioid receptor protein (U02083, RNU02083), rat-derived human-derived RANTES receptor protein (L10918, HUMRANTES),  $\kappa$  -Opioid receptor protein (U00442, U00442), human-derived human Burkitt's lymphoma-derived receptor protein with an  $a_{_{_{\phantom{1}}}}B$  receptor protein (L08609, RATAADRE01), human-derived human-derived receptor protein with an unknown ligand corresponding to or near the first membrane-spanning the DNA seguence coding for the amino acid seguence somatostatin 3 receptor protein (M96738, HUMSSTR3X),  $a_{
m j} B$  receptor protein (M91466, RATA2BAR) and the like receptor protein with an unknown ligand (HUMRDCLA), neuromedin B receptor protein (M73482, HUMNMBR), unknown ligand (X68149, HSBLR1A), human-derived human-derived muscarinic acetylcholine receptor Protein (X15266, HSHM4), rat-derived adrenaline (M84605, HUMOPIODRE), rat-derived adrenaline

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sequence coding for the amiho acid sequence corresponding to or The nucleotide sequence represented by SEQ ID NO: 2 (HS-2) is a nucleotide sequence which is complementary to the nucleotide seguence (Figure 2) highly homologous to the DNA near the sixth membrane-spanning domain of known G protein coupled receptor proteins such as mouse-derived receptor

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protein with an unknown ligand (M80481, MUSGIR), human-derived inknown ligand (X61496, RNCGPCR), human-derived somatostatin 4 adenosine A2 receptor protein (S46950, S46950), mouse-derived rat-derived neuromedin K receptor protein (J05189, RATNEURA), rat-derived adenosine Al receptor protein (M69045, RATALARA), bombesin receptor protein (L08893, HUMBOMB3S), human-derived rat-derived adenosine A3 receptor protein (M94152, RATADENRE human-derived neurokinin A receptor protein (M57414, HUMNEKA) receptor protein (M31670, RATGNRHA) and the like [Figure 2]. receptor protein with an unknown ligand (D21061, MUSGPCR), (S86390, S86371S4), rat-derived receptor protein with an HUMSTRILA), human-derived neurokinin 3 receptor protein receptor protein (L07061, HUMSSTR42), rat-derived GnRH human-derived somatostatin 1 receptor protein (M81829, mouse-derived TRH receptor protein (S43387, S43387),

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mouse-derived 6 -opioid receptor protein (L11065), rat-derived subtype 3 (L08893), mouse-derived substance K receptor protein (X62933), mouse-derived substance P receptor protein (X62934), The nucleotide sequence represented by SEQ ID NO: 5  $\mu$  -opicid receptor protein (D16349), mouse-derived bradykining sequence corresponding to or near the third membrane-spanning protein (M35328), human-derived neuromedin B receptor protein rat-derived endothelin receptor protein (M60786), rat-derived receptor protein with an unknown ligand (X61496), rat-derived receptor protein with an unknown ligand (L04672), rat-derived (Figure 3: 3A) or the nucleotide seguence represented by SEQ receptor protein (M59967), mouse-derived bombesin receptor (M73482), human-derived gastrin releasing peptide receptor protein (M73481), human-derived bombesin receptor protein homologous to the DNA sequence coding for the amino acid B2 receptor protein (X69676), rat-derived bradykinin B2 ID NO: 6 (Figure 3: 3B) is a nucleotide seguence highly as mouse-derived  $\kappa$  -opioid receptor protein (L11064), domain each of known G protein coupled receptors such rat-derived neurokinin 3 receptor protein (J05189),

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receptor protein with an unknown ligand (X59249), rat-derived receptor protein with an unknown ligand (L09249),

and the like [Figure 3]. human-derived receptor protein with an unknown ligand (003642) human-derived receptor protein with an unknown ligand (M31210), mouse-derived receptor protein with an unknown ligand (P30731),

15 20 S as mouse-derived angiotensin II receptor protein (L32840), domain each of known G protein coupled receptors such sequence corresponding to or near the third membrane-spanning ID NO: 3 (Figure 4: 3D) is a nucleotide sequence highly homologous to the DNA sequence coding for the amino acid (Figure 4: 3C) or the nucleotide sequence represented by SEQ mouse-derived low affinity interleukin 8 receptor protein rat-derived cholecystokinin a receptor protein (M88096), rat-derived angiotensin Ib receptor protein (X64052), protein (M60626) and the like [Figure 4]. protein (S4665), human-derived N-formylpeptide receptor protein (x65858), mouse-derived C5a anaphylatoxin receptor (M73969), human-derived high affinity interleukin 8 receptor human-derived cholecystokinin b receptor protein (L04473), rat-derived cholecystokinin b receptor protein (M99418), human-derived angiotensin Ia receptor protein (M91464), rat-derived angiotensin receptor protein subtype (M90065). The nucleotide sequence represented by SEQ ID NO: 7

human galanin receptor (HUMGALAREC), rat  $\alpha$  -1B-adrenergic corresponding to or near the second membrane-spanning domain receptor (RATADR1B), human  $\beta$  -1-adrenergic receptor each of known G protein coupled receptors such as the DNA sequence coding for the amino acid sequence (Figure 7: T2A) is a nucleotide sequence highly homologous to serotonin receptor-2 (MMSERO), rat a -lA-adrenergic receptor receptor-D5 (HUMD1B), human serotonin receptor 5HT1E human cholecystokinin A receptor (HUMCCKAR), human dopamine receptor-3 (HUMSSTR3Y), human gastrin receptor (HUMGARE), human somatostatin receptor-2 (HUMSRI2A), human somatostatin receptor (HUMOPIODRE), bovine substance K receptor (BTSKR), (HUMADRB1), rabbit IL-8 receptor (RABIL8RSB), human opioid (HUM5HTlE), human dopamine receptor D4 (HUMD4C), mouse The nucleotide sequence represented by SEQ ID NO: 10

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the like [Figure 7]. (RATADRA1A), rat histamine H2 receptor (S57565) and

5 J coupled receptors such as mouse-derived  $\kappa$  -opioid or near the sixth membrane-spanning domain of known G protein sequence coding for the amino acid sequence corresponding to nucleotide sequence (Figure 5) highly homologous to the DNA is a nucleotide sequence which is complementary to the represented by SEQ ID NO: 9 (complementary to 6B of Figure 5) (complementary to 6A of Figure 5) or the nucleotide sequence receptor protein (L11064), mouse-derived  $\delta$  -opioid receptor The nucleotide sequence represented by SEQ ID NO: 8

15 mouse-derived bombesin receptor protein (M35328), (X69676), rat-derived bradykinin B2 receptor protein (M59967), (D16349), mouse-derived bradykinin B2 receptor protein mouse-derived substance P receptor protein (X62934), human-derived gastrin releasing peptide receptor protein human-derived neuromedin B receptor protein (M73482), (L08893), mouse-derived substance K receptor protein (x62933), (M73481), human-derived bombesin receptor protein subtype 3

protein (L11065), rat-derived  $\mu$  -opioid receptor protein

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ä 25 rat-derived receptor protein with an unknown ligand (L09249), rat-derived receptor protein with an unknown ligand (X59249), rat-derived receptor protein with an unknown ligand (x61496), rat-derived receptor protein with an unknown ligand (L04672), rat-derived endothelin receptor protein (M60786), rat-derived neurokinin 3 receptor protein (J05189), human-derived receptor protein with an unknown ligand (M31210) mouse-derived receptor protein with an unknown ligand (P30731), and the like [Figure 5]. human-derived receptor protein with an unknown ligand (U03642)

sequence corresponding to or near the sixth membrane-spanning highly homologous to the DNA sequence coding for the amino acid which is complementary to the nucleotide sequence (Figure 6) (complementary to 6C of Figure 6) is a nucleotide sequence mouse-derived angiotensin II receptor protein (L32840), domain of known G protein coupled receptors such as The nucleotide sequence represented by SEQ ID NO: 4

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rat-derived angiotensin Ib receptor protein (X64052), rat-derived angiotensin receptor protein subtype (M90065), human-derived angiotensin Ia receptor protein (M91464), rat-derived cholecystokinin a receptor protein (M88096), rat-derived cholecystokinin b receptor protein (M99418), human-derived cholecystokinin B receptor protein (L04473), mouse-derived low affinity interleukin B receptor protein (M73969), human-derived high affinity interleukin B receptor protein (X65858), mouse-derived C5a anaphylatoxin receptor protein (S46665), human-derived N-formylpeptide receptor protein (M60626) and the like [Figure 6].

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somatostatin receptor (HUMSST28A), rat receptor with an unknown The nucleotide sequence represented by SEQ ID NO: 11 (RRVTlAIIR), human muscarinic acetylcholine receptor (HSHM4), delta-opioid receptor (S66181), human somatostatin receptor-3 ligand (RNGPROCR), mouse somatostatin receptor-1 (MUSSRIIA), receptor (HUMGARE), rat cholecystokinin receptor (RATCCKAR), complementary to the nucleotide sequence (Figure 8) highly rat Al adenosine receptor (RAT1DREC), porcine angiotensin human dopamine receptor (SS8541), human gastrin releasing homologous to the DNA sequence coding for the amino acid membrane-spanning domain each of known G protein coupled human eta -1 adrenergic receptor (HUMDRB1), human gastrin peptide receptor (HUMGRPR), mouse GRP/bombesin receptor receptor (PIGA2R), rat serotonin receptor (RAT5HTRTC), receptors such as human galanin receptor (HUMGALAREC), (MUSGRPBOM), rat vascular type 1 angiotensin receptor rat receptor with an unknown ligand (S59748), human (Figure 8: T7A ) is a nucleotide sequence which is human a -Al-adrenergic receptor (HUMAlAADR), mouse sequence corresponding to or near the seventh (HUMSSTR3Y) and the like [Figure 8].

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The nucleotide sequence represented by SEQ ID NO: 12 (Figure 9: TM1-A2) is a nucleotide sequence highly homologous to the DNA sequence coding for the amino acid sequence within the first membrane-spanning (transmembrane) domain each of known G protein coupled receptors such as

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mouse-derived bradykinin B<sub>2</sub> receptor (MUSBB2R), bovine-derived substance K receptor (BTSKR), bovine-derived endothelin ET<sub>B</sub> receptor (BOVEETBR), human-derived neuropeptide Y receptor (MUNDEREC), human-derived prostaglandin E<sub>2</sub> receptor (HUMPIR), human-derived ~ -opioid receptor (HSU11053), rat-derived melanocortin 3 receptor (RRMC3RA), human-derived melanocortin 3 receptor (RRMC3RA), human-derived melanocortin receptor (HUMMR), mouse-derived bombesin/GRP receptor (MUSGRPBOM), rat-derived cholecystokinin B receptor (RATCCKAR) and the like [Figure 9].

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The nucleotide sequence represented by SEQ ID NO: 13 is complementary to the nucleotide sequence (Figure 10) highly neuromedin K receptor (RAINEURA), dog-derived gastrin receptor membrane-spanning domain of known G protein coupled receptors such as human-derived cholecystokinin receptor (HUMCCKR), homologous to the DNA sequence coding for the amino acid human-derived vasopressin receptor (HUMV2R), rat-derived sequence corresponding to or near the end of the third (DOGGSTRN), rat-derived serotonin receptor (RATSHTSA), human-derived opioid (presumed) receptor (HUMOPIODRE), human-derived cholecystokinin B receptor (HUMCCKBGR), mouse-derived  $a_2$  -adrenaline receptor (MUSALP2ADA), (Figure 10: TM3-B2) is a nucleotide sequence which human-derived adenosine  ${\sf A}_1$  receptor (HUMADORALX), mouse-derived melanocortin 5 receptor (MMGMC5R), mouse-derived bombesin/GRP receptor (MUSGRPBOM),

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human-derived TRH receptor (HSTRHREC) and the like [Figure 10].

The nucleotide sequence represented by SEQ ID NO: 14
(Figure 11: TM3-C2) is a nucleotide sequence highly homologous to the DNA sequence coding for the amino acid sequence corresponding to or near the end of the third membrane-spanning domain of known G protein coupled receptors such as human-derived neurokinin 3 receptor (HUMNK3R), human-derived oxytocin receptor (HSMRNAOXY), guinea pig-derived

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rat-derived cholecystokinin A receptor (RATCCKAR),

like [Figure 11]. rat-derived cholecystokinin A receptor (RATCCKAR) and the (HUMARB3A), human-derived prostacyclin receptor (HUMHPR), galanin receptor (HUMGALAREC), human-derived serotonin receptor with an unknown ligand (HUMOPIODRE), human-derived mouse-derived substance P receptor (MMSUBPREC), human-derived receptor (HSS31G), human-derived  $eta_{f \gamma}$ -adrenaline receptor cholecystokinin A receptor with an unknown ligand (CFGPCR4), cholecystokinin A receptor (S68242), dog-derived

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receptor (HSMRNAOXY), rat-derived cholecystokinin A receptor neuropeptide Y1 receptor (MMNPY1CDS), human-derived oxytocin G protein coupled receptors such as human-derived neurokinin A human-derived endothelin receptor (HUMETSR), mouse-derived canine-derived receptor RDC5 with an unknown ligand (CFGPCR8), human-derived adenosine  $A_2$  receptor (HUMA2XXX), human-derived  $eta_2^{}$ -adrenaline receptor (HUMADRBR), human-derived opioid (presumed) receptor (HUMOPIODRE), mouse-derived bombesin/GRP receptor (MUSGRPBOM), sequence within the sixth membrane-spanning domain of known homologous to the DNA sequence coding for the amino acid (RATCCKAR) and the like [Figure 12]. receptor (HUMNEKAR), human-derived substance P receptor is complementary to the nucleotide sequence (Figure 12) highly (Figure 12: TM6-E2) is a nucleotide sequence which (HUMSUBPRA), rat-derived substance K receptor (RATSKR), The nucleotide sequence represented by SEQ ID NO: 15

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receptor A (HUMIL8RA), human-derived dopamine D2 receptor a -Al adrenergic receptor (HUMALAADR), human-derived IL8 human-derived IL8 receptor B (HUMINTLEU8), human-derived receptor (HUMTSHX), human-derived neurokinin A receptor of known G protein coupled receptors such as human-derived TSH corresponding to or near the second membrane-spanning domain to the DNA sequence coding for the amino acid sequence (HSDD2), human-derived angiotensin type I receptor (HUMANTIR), (Figure 13: TM2F18) is a nucleotide sequence highly homologous (HUMNEKAR), human-derived FMLP receptor (HUMFMLP), The nucleotide sequence represented by SEQ ID NO: 16

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(HSU07882) and the like [Figure 13]. TRH receptor (HSTRHREC), human-derived delta-opioid receptor human-derived somatostatin receptor (HUSOMAT), human-derived

տ domain of known G protein coupled receptors such as sequence corresponding to or near the sixth membrane-spanning homologous to the DNA sequence coding for the amino acid complementary to the nucleotide sequence (Figure 14) highly (Figure 14: TM6R21) is a nucleotide sequence which is The nucleotide sequence represented by SEQ ID NO: 17

10 human-derived  $oldsymbol{eta}$  -adrenergic receptor (HSBAR), human-derived receptor (HUMETNIR), human-derived histamine  $H_2$  receptor neurokinin A receptor (HUMNEKAR), human-derived endothelin-1 (HUMHISH2R), human-derived a -Al adrenergic receptor (HUMALAADR), human-derived IL8 receptor A (HUMIL8RA),

25 20 5 neurotensin receptor (HSNEURA) and the like [Figure 14]. human-derived cholecystokinin receptor (HUMCCKR), human-derived human-derived somatostatin receptor subtype 3 (HUMSSTR3X), (HUMBK2A), human-derived FMLP-related receptor II (HUMFMLPX), A2 receptor (HUMA2XXX), human-derived bradykinin receptor BK-2 neuropeptide Y receptor (HUMNEUYREC), human-derived adenosine human-derived dopamine D2 receptor (HSDD2), human-derived receptor (HUMSUBPRA), human-derived 5-HT1D serotonin receptor neurokinin 1 receptor (HUMNKIRX), human-derived substance P human-derived neuromedin B receptor (HUMNMBR), human-derived (HUM5HT1DA), human-derived formylpeptide receptor (HUMPFPR2A),

30 of known G protein coupled receptors such as human-derived human-derived angiotensin II receptor (HUMANTIR), ET receptor (\$44866), human-derived C5A receptor (HUMC5AAR), (S70057), human-derived ET receptor (S67127), human-derived galanin receptor (HUMGALAREC), human-derived CCK-B receptor corresponding to or near the third membrane-spanning domain to the DNA sequence coding for the amino acid sequence (Figure 15: S3A) is a nucleotide sequence highly homologous The nucleotide sequence represented by SEQ ID NO: 18

35 neurotensin receptor (HSNEURA), human-derived GRP receptor human-derived bradykinin receptor (HUMBK2R), human-derived (HUMGRPR), human-derived somatostatin 5 receptor (HUMFSRS),

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human-derived IL-8 receptor (HUMIL8RA), human-derived neurokinin 2 (neurokinin A) receptor (HUMNEKAR) and the like [Figure 15].

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human-derived ET $_{
m B}$  receptor (S44866), human-derived CSA receptor The nucleotide sequence represented by SEQ ID NO: 19 sequence corresponding to or near the sixth membrane-spanning (HUMCSAAR), human-derived angiotensin II receptor (HUMANTIR), neurokinin 2 (neurokinin A) receptor (HUMNEKAR) and the like complementary to the nucleotide sequence (Figure 16) highly (HUMGRPR), human-derived somatostatin 5 receptor (HUMFSRS), domain of known G protein coupled receptors such as humanhuman-derived bradykinin receptor (HUMBK2R), human-derived neurotensin receptor (HSNEURA), human-derived GRP receptor derived galanin receptor (HUMGLAREC), human-derived CCK-B nomologous to the DNA sequence coding for the amino acid receptor (S70057), human-derived ET  $_{
m A}$  receptor (S67127), human-derived IL-8 receptor (HUMIL8RA), human-derived (Figure 16: S6A) is a nucleotide sequence which is [Figure 16].

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The above-mentioned abbreviations in the parentheses are the identifiers (or reference numbers) which are shown when GenBank/EMBL Data Bank is searched using a DNASIS Gene/Protein Sequence Data Base (CD019; Hitachi Software Engineering, Japan) and are usually called "Accession Numbers" or "Entry Names". HTRHR is, however, the sequence as described in Japanese Patent Application No. Hei 5-286986 (or No. 286986/1993) (EPA 638645).

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The DNA (or nucleotides) of the present invention may be manufactured by DNA synthetic methods which are known per se or by methods similar thereto. The DNA (or nucleotides) of the present invention may be an oligonucleotide sequence having 8 to 60 base residues, preferably 12 to 50 base residues, more preferably 15 to 40 residues and most preferably 18 to 30 residues.

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Among the DNAs of the present invention, the DNA having the nucleotide sequence represented by SEQ ID NO: 1 or

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present in the nucleotide sequence which is commonly present in the nucleotide sequence of the DNA encoding the amino acid sequence corresponding to or near the first membrane-spanning domain of the above-mentioned known G protein coupled receptor protein. Therefore, it can be complementarily bonded (i.e. is hybridizable) with RNA or DNA (including genome DNA, cDNA) coding for the amino acid sequence corresponding to or near the first membrane-spanning domain q known or unknown G protein coupled receptor proteins and, furthermore, it can be complementarily bonded (i.e. is hybridizable) with nucleotide sequences encoding other membrane-spanning domains as well.

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The DNA having a nucleotide sequence represented by SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 14 or SEQ ID NO:18 is a nucleotide sequence which is commonly present in the nucleotide sequence of the DNA coding for the amino acid sequence corresponding to or near the third membrane-spanning domain of the above-mentioned known G protein coupled receptor protein. Therefore, it can be complementarily bonded with RNA or DNA (including genome DNA, cDNA) coding for the part corresponding to or near the third membrane-spanning domain of known or unknown G protein coupled receptor proteins and, furthermore, it can be complementarily bonded with nucleotide sequences encoding other membrane-spanning domains as well.

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The DNA having a nucleotide sequence represented by SEQ ID NO: 10 or SEQ ID NO: 16 is a nucleotide sequence which is commonly present in the nucleotide sequence of the DNA coding for the amino acid sequence corresponding to or near the second membrane-spanning domain of the above-mentioned known G protein coupled receptor protein. Therefore, it can be complementarily bonded with RNA or DNA (including genome DNA, cDNA) coding for the part coxresponding to or near the second membrane-spanning domain of known or unknown G protein coupled receptor proteins and, furthermore, it can be complementarily bonded with nucleotide sequences encoding other membrane-spanning domains as well.

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coding for the amino acid sequence corresponding to or near cDNA) coding for the part corresponding to or near the sixth G protein coupled receptor protein. Therefore, it can be which is commonly present in the nucleotide sequence of the DNA receptor proteins and, furthermore, it can be complementarily membrane-spanning domain of known or unknown G protein coupled complementarily bonded with RNA or DNA (including genome DNA) the sixth membrane-spanning domain of the above-mentioned known NO: 15, SEQ ID NO: 17 or SEQ ID NO: 19 is a nucleotide sequence SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID membrane-spanning domains as well. bonded with nucleotide sequences encoding other The DNA having a nucleotide sequence represented by

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15 20 and, further more, it can be complementarily bonded with the part corresponding to or near the seventh membrane-spanning membrane-spanning domain of the above-mentioned known G protein amino acid sequence corresponding to or near the seventh present in the nucleotide sequence of the DNA coding for the SEQ ID NO: 11 is a nucleotide seguence which is commonly nucleotide sequences encoding other transmembrane domains domain of known or unknown G protein coupled receptor proteins bonded with RNA or DNA (including genome DNA, cDNA) coding for coupled receptor protein. Therefore, it can be complementarily The DNA having a nucleotide sequence represented by

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coupled receptor protein. Therefore, it can be complementarily membrane-spanning domain of the above-mentioned known G protein present in the nucleotide sequence of the DNA coding for the SEQ ID NO: 13 is a nucleotide sequence which is commonly nucleotide sequences encoding other membrane-spanning domains and, furthermore, it can be complementarily bonded with domain of known or unknown G protein coupled receptor proteins the part corresponding to or near the third membrane-spanning bonded with RNA or DNA (including genome DNA, cDNA) coding for amino acid sequence corresponding to or near the third The DNA having a nucleotide sequence represented by

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reaction (hereinafter, sometimes referred to as PCR). invention can be used as DNA primers for a polymerase chain Accordingly, the DNAs (or nucleotides) of the present

տ (i) a polymerase chain reaction is carried out by

(1) a small amount of DNA (or DNA fragment(s)) which codes for G protein coupled receptor protein, said DNA (or DNA fragment(s)) acting as a template,

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(2) at least one DNA primer selected from the group nucleotide sequence represented by SEQ ID NO: 3, DNA represented by SEQ ID NO: 1, DNA primers having a consisting of DNA primers having a nucleotide sequence nucleotide sequence represented by SEQ ID NO: 7, DNA ID NO: 5, DNA primers having a nucleotide sequence primers having a nucleotide sequence represented by SEQ primers having a nucleotide sequence represented by SEQ represented by SEQ ID NO: 6, DNA primers having a represented by SEQ ID NO: 18 and primers having a nucleotide sequence represented by SEQ nucleotide sequence represented by SEQ ID NO: 14, DNA represented by SEQ ID NO: 12, DNA primers having a ID NO: 10, DNA primers having a nucleotide sequence ID NO: 16 and DNA primers having a nucleotide sequence

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(ii) a polymerase chain reaction is carried out by mixing (3) at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence primers having a nucleotide sequence represented by SEQ nucleotide sequence represented by SEQ ID NO: 11, DNA ID NO: 8, DNA primers having a nucleotide sequence primers having a nucleotide sequence represented by SEQ nucleotide sequence represented by SEQ ID NO: 4, DNA represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 19; or represented by SEQ ID NO: 9, DNA primers having a ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a

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 a small amount of DNA (or DNA fragment(s)) coding for G protein coupled receptor protein, said DNA (or DNA fragment(s)) acting as a template,

(2) at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 12 and

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(3) at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 13

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so that it is possible to amplify the target DNA (or DNA fragment(s)) coding for said receptor protein.

primer selected from the group consisting of DNA primers having a nucleotide seguence represented by SEQ ID NO: 2, DNA primers NO: 8, DNA primers having a nucleotide sequence represented by having a nucleotide sequence represented by SEQ ID NO: 4, DNA represented by SEQ ID NO: 11, DNA primers having a nucleotide chain) of template RNA or DNA (or fragment(s) thereof) coding When the PCR is carried out using at least one DNA whereupon an elongation of the - chain (minus chain) proceeds sequence represented by SEQ ID NO: 15, DNA primers having a the nucleotide sequence at the 3'-side of the + chain (plus NO: 19, said DNA primer(s) is(are) bonded (hybridized) with primers having a nucleotide sequence represented by SEQ ID primers having a nucleotide sequence represented by SEQ ID for the sixth membrane-spanning domain or other membranenucleotide sequence represented by SEQ ID NO: 17 and DNA SEQ ID NO: 9, DNA primers having a nucleotide sequence spanning domains of G protein coupled receptor protein in the 5' - 3' direction.

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When the PCR is carried out using at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 11, said DNA primer is bonded with the nucleotide sequence at the 3'-side of the + chain (plus chain) of template RNA or DNA (or fragment(s) thereof) coding for the seventh membrane-spanning domain or other membrane-spanning domains of the G protein

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coupled receptor protein whereupon an elongation of the - chain (minus chain) proceeds in the 5'  $\rightarrow$  3' direction,

When the PCR is carried out using at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 12, said DNA primer is bonded with the nucleotide sequence at the 3'-side of the - chain (minus chain) of template RNA DNA (or fragment(s) thereof) coding for the first membrane-spanning domain or other membrane-spanning domains of G protein coupled receptor protein whereupon an elongation of the + chain (plus chain) proceeds in the 5' - 3' direction.

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When the PCR is carried out using at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 10 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 10 and DNA primer is bonded with the nucleotide sequence at the 3'-side of the - chain (minus chain) of template RNA or DNA (or fragment(s) thereof) coding for the second membrane-spanning domain or other membrane-spanning domains of G protein coupled receptor protein whereupon an elongation of the + chain (plus chain) proceeds in the 5' - 3' direction.

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When the PCR is carried out using at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 3, DNA primers having a nucleotide sequence represented by SEQ ID NO: 5, DNA primers having a nucleotide sequence represented by SEQ ID NO: 6, DNA primers having a nucleotide sequence represented by SEQ ID NO: 7, DNA primers having a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 18, said DNA primer is bonded with the nucleotide sequence at the 3'-side of the - chain (minus chain) of template RNA or DNA (or fragment(s) thereof) coding for the third membrane-spanning domain or other membrane-spanning domains of G protein coupled receptor protein whereupon an elongation of the + chain (plus chain) proceeds in the 5' - 3' direction.

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nucleotide sequence represented by SEQ ID NO: 12 and

- ② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO:11;
- 5 (C) a method of amplifying a DNA coding for the G protein coupled receptor protein (e.g., from the second to sixth membrane-spanning (transmembrane) domains or other segments of the G protein coupled receptor protein), characterized in that a polymerase chain reaction is carried out by mixing
- ② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 10 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 16 and

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- ② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19;
- (D) a method of amplifying a DNA coding for the G protein coupled receptor protein (e.g., from the second to seventh membrane-spanning (transmembrane) domains or other segments of the G protein coupled receptor protein), characterized in that a polymerase chain reaction is carried out by mixing
- ${\mathbb O}$  a DNA coding for the G protein coupled receptor protein, said DNA acting as a template,
- ② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 10 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 16 and

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at least one DNA primer selected from the group

Accordingly, when the DNA primers having nucleotide sequences represented by any of SEQ ID NO: 1 to SEQ ID NO: 19 of the present invention are used in combination each other, DNA (or DNA fragment(s)) coding for G protein coupled receptor protein can be successfully amplified.

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One embodiment of the present invention provides:
(A) a method of amplifying DNA coding for the G protein coupled receptor protein (e.g., from the first to sixth membrane-spanning (transmembrane) domains or other segments of the G protein coupled receptor protein), characterized in that a polymerase chain reaction is carried out by mixing

- $\ensuremath{\mathbb{O}}$  a DNA coding for the G protein coupled receptor protein, said DNA acting as a template,
- ② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 12 and

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- ② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence
- ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19;
  (B) a method of amplifying DNA coding for the G protein coupled receptor protein (e.g., from the first to seventh membrane-spanning (transmembrane) domains or other segments of the G protein coupled receptor protein), characterized in that a polymerase chain reaction is carried out by mixing

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- ${\mathbb O}$  a DNA coding for the G protein coupled receptor protein, said DNA acting as a template,
- ② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a

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consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 11;

(E) a method of amplifying a DNA coding for the G protein coupled receptor protein (e.g., from the third to sixth membrane-spanning (transmembrane) domains or other segments of the G protein coupled receptor protein), characterized in that a polymerase chain reaction is carried out by mixing

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 $\ensuremath{\mathbb{O}}$  a DNA coding for the G protein coupled receptor protein, said DNA acting as a template,

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© at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 3, DNA primers having a nucleotide sequence represented by SEE ID NO: 5, DNA primers having a nucleotide sequence represented by SEQ ID NO: 6, DNA primers having a nucleotide sequence represented by SEQ ID NO: 7, DNA primers having a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 18 and

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© at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19;

(F) a method of amplifying a DNA coding for the G protein coupled receptor protein (e.g., from the third to seventh membrane-spanning (transmembrane) domains or other segments of the G protein coupled receptor protein), characterized in that a polymerase chain reaction is carried out by mixing

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① a DNA coding for the G protein coupled receptor protein, said DNA acting as a template,

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at least one DNA primer selected from the group

consisting of DNA primers having a nucleotide sequence

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represented by SEQ ID NO: 3, DNA primers having a nucleotide sequence represented by SEQ ID NO: 5, DNA primers having a nucleotide sequence represented by SEQ ID NO: 6, DNA primers having a nucleotide sequence represented by SEQ ID NO: 7, DNA primers having a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 18 and

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② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO:11; and

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(G) a method of amplifying a DNA coding for the G protein coupled receptor protein (e.g., from the first to third membrane-spanning (transmembrane) domains or other segments of the G protein coupled receptor protein), characterized in that a polymerase chain reaction is carried out by mixing

 $\ensuremath{\mathbb{O}}$  a DNA coding for the G protein coupled receptor protein, said DNA acting as a template,

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© at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 12 and

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③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 13.

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An example of more preferred combination of the DN primers in the amplification according to the above-mentioned (A) includes a combination of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 1 with a DNA primer having a nucleotide sequence represented by SEQ ID NO: 2 and the like.

An example of more preferred combination of the DNA primers in the amplification according to the above-mentioned (D) includes a combination of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 10 with a DNA primer having a nucleotide sequence represented by SEQ ID NO: 11 and the

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An example of more preferred combination of the DNA

primers in the amplification according to the above-mentioned (E) includes:

sequence represented by SEQ ID NO: 6 with a DNA primer having a nucleotide sequence represented by SEQ ID NO: 8 or a DNA primer having a nucleotide sequence represented by SEQ ID NO: 9; represented by SEQ ID NO: 5 or a DNA primer having a nucleotide (i) a combination of a DNA primer having a nucleotide sequence

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sequence represented by SEQ ID NO: 7 with a DNA primer having a represented by SEQ ID NO: 3 or a DNA primer having a nucleotide nucleotide sequence represented by SEQ ID NO: 4 and the (ii) a combination of a DNA primer having a nucleotide sequence

a nucleotide sequence represented by SEQ ID NO: 13 and the sequence represented by SEQ ID NO: 12 with a DNA primer having primers in the amplification according to the above-mentioned (G) includes a combination of a DNA primer having a nucleotide An example of more preferred combination of the DNA

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20 R. K. et al., Science, 239:487-491(1988). When RNA is used as a template, PCR amplification may be upon the type of target DNA and other factors. by the method described in Saiki, R. K. et al., Science, with known PCR techniques. For example, it may be carried out carried out, for example, by the method described in Saiki in the PCR amplification may be suitably selected depending 2'-deoxy-7-deazaguanosine triphosphate or inosine, etc. reaction cycles, enzyme such as DNA polymerase, addition of 239:487-491 (1988). Temperature, time, buffer, number of The amplification may be carried out in accordance

corresponding to or near the first membrane-spanning domain nucleotide sequence represented by SEQ ID NO: 10 or SEQ ID represented by SEQ ID NO: 1 or SEQ ID NO: 12 of the present of the G protein coupled receptor protein; the DNA having a the - chain of the DNA coding for the amino acid sequence (hybridized) with the nucleotide sequence at the 3'-side of invention can be selectively and complementarily bonded Moreover, the DNA having a nucleotide sequence

These screening methods for DNA (or DNA fragment(s))

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15 10 25 20 30 υ 35 nucleotide sequence at the 3'-side of the - chain of the DNA SEQ ID NO: 14 or SEQ ID NO: 18 of the present invention can be SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, protein; the DNA having a nucleotide sequence represented membrane-spanning domain of the G protein coupled receptor the amino acid sequence corresponding to or near the second complementarily bonded (hybridized) with the nucleotide coding for the amino acid sequence corresponding to or near selectively and complementarily bonded (hybridized) with the sequence at the 3'-side of the - chain of the DNA coding for NO: 16 of the present invention can be selectively and of the G protein coupled receptor protein; the DNA having of the + chain of the DNA coding for the amino acid sequence of the present invention can be selectively and complementarily SEQ ID NO: 9, SEQ ID NO: 15, SEQ ID NO: 17 or SEQ ID NO: 19 the third membrane-spanning domain of the G protein coupled sequence corresponding to or near the third membrane-spanning a nucleotide sequence represented by SEQ ID NO: 11 of the corresponding to or near the sixth membrane-spanning domain bonded (hybridized) with the nucleotide seguence at the 3'-side represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 8, receptor protein; the DNA having a nucleotide sequence sequence corresponding to or near the third membrane-spanning 3'-side of the + chain of the DNA coding for the amino acid of the present invention can be selectively and complementarily DNA having a nucleotide sequence represented by SEQ ID NO: 13 domain of the G protein coupled receptor protein; and the 3'-side of the + chain of the DNA coding for the amino acid bonded (hybridized) with the nucleotide sequence at the present invention can be selectively and complementarily encoding part or all of the polypeptide sequence of G protein probe for screening DNA libraries for DNA (or DNA fragment(s)) accordingly, said DNA is also advantageously useful as a domain of the G protein coupled receptor protein and, bonded (hybridized) with the nucleotide sequence at the coupled receptor proteins.

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encoding part or all of the polypeptide sequence of G protein coupled receptor proteins from the DNA library by using as a reagent, because it can be used as a probe the DNA of the present invention may be carried out according to DNA cloning methods known per se by those of skill in the art or methods similar thereto. Especially when the DNA of the present invention is used as a DNA primer for the PCR, both amplification and screening of the DNA (or DNA fragment) coding for the G protein coupled receptor protein can be conducted in a single step.

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Thus, when the DNAs of the present invention are suitably combined and used as the DNA primer for the PCR, said DNA primer(s) is(are) bonded (hybridized) with RNA or DNA (or fragment(s) thereof) encoding the amino acid sequence of the first membrane-spanning (transmembrane) domain, the second membrane-spanning domain, the third membrane-spanning domain, the sixth membrane-spanning domain, the seventh membrane-spanning domain or other membrane-spanning domains of G protein coupled receptor proteins to amplify, for example, O RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of from the first membrane-spanning to the sixth membrane-spanning domains of G protein coupled receptor proteins,

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RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of from the first membrane-spanning to the seventh membrane-spanning domains of G protein coupled receptor proteins,

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® RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of from the third membrane-spanning to the sixth membrane-spanning domains of G protein coupled receptor proteins.

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 RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of from the third membrane-spanning to the seventh membrane-spanning domains of G protein coupled receptor proteins,

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⑤ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of from the second membrane-spanning to the

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sixth membrane-spanning domains of G protein coupled receptor proteins or RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of other domains thereof,

© RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of from the second membrane-spanning to the seventh membrane-spanning domains of G protein coupled receptor proteins,

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© RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of from the first membrane-spanning to the thir membrane-spanning domains of G protein coupled receptor proteins or

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® RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of other domains of G protein coupled receptor proteins.

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- Through using the DNA primer according to the present invention, therefore, selective amplifications of:

  ① RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence covering from the first membrane-spanning domain to the sixth membrane-spanning domain of G protein coupled receptor proteins;
- © RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence covering from the first membrane-spanning domain to the seventh membrane-spanning domain of G protein coupled receptor proteins;
- acid sequence covering from the third membrane-spanning domaint to the sixth membrane-spanning domain of G protein coupled receptor proteins;
- ⑤ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence covering from the second membrane-spanning domain to the sixth membrane-spanning domain of G protein coupled receptor proteins or RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence covering other areas thereof,

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to the seventh membrane-spanning domain of G protein coupled acid sequence covering from the second membrane-spanning domain RNA or DNA (or fragment(s) thereof) coding for the amino

acid sequence covering from the first membrane-spanning domain to the third membrane-spanning domain of G protein coupled receptor proteins; receptor proteins; and the like, RNA or DNA (or fragment(s) thereof) coding for the amino

from DNA libraries can be successfully achieved.

Among the DNA primers of the present invention,

SEQ ID NO: 1 or SEQ ID NO: 2; with the combination of a DNA primer having a nucleotide sequence represented by

5 20 of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 2, a DNA primer having a nucleotide sequence having a nucleotide sequence represented by SEQ ID NO: 15, nucleotide sequence represented by SEQ ID NO: 9, a DNA primer sequence represented by SEQ ID NO: 8, a DNA primer having a represented by SEQ ID NO: 4, a DNA primer having a nucleotide at least one DNA primer selected from the group consisting

a DNA primer having a nucleotide sequence represented by SEQ spanning domain to the sixth membrane-spanning domain or other amplifying a broad area covering from the first membranerepresented by SEQ ID NO: 19; ID NO: 17 and a DNA primer having a nucleotide sequence is, unlike conventional primers, capable of selectively

domains of G protein coupled receptor proteins. Among the DNA primers of the present invention, the

combination of

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 ${\mathbb Q}$  a DNA primer having a nucleotide sequence represented by SEQ ID NO: 1 or SEQ ID NO: 12; with SEQ ID NO: 11; a DNA primer having a nucleotide sequence represented by

<u>3</u>5 amplifying a broad area covering from the first membraneis, unlike conventional primers, capable of selectively spanning domain to the seventh membrane-spanning domain or other domains of G protein coupled receptor proteins.

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Among the DNA primers of the present invention, the

SEQ ID NO: 10 or SEQ ID NO: 16; with  $oldsymbol{\mathbb{Q}}$  a DNA primer having a nucleotide sequence represented by combination of

- ö v  ${f @}$  at least one DNA primer selected from the group consisting of a DNA primer having a nucleotide sequence represented by SEQ sequence represented by SEQ ID NO: 9, a DNA primer having a represented by SEQ ID NO: 8, a DNA primer having a nucleotide by SEQ ID NO: 4, a DNA primer having a nucleotide sequence ID NO: 2, a DNA primer having a nucleotide sequence represented a DNA primer having a nucleotide sequence represented by having a nucleotide sequence represented by SEQ ID NO: 17 and nucleotide sequence represented by SEQ ID NO: 15, a DNA primer
- 5 amplifying a broad area covering from the second membraneis, unlike conventional primers, capable of selectively SEQ ID NO: 19; other domains of G protein coupled receptor proteins. spanning domain to the sixth membrane-spanning domain or Among the DNA primers of the present invention, the
- 20  $oldsymbol{\mathbb{Q}}$  a DNA primer having a nucleotide sequence represented by SEQ ID NO:10 or SEQ ID NO:16; with combination of a DNA primer having a nucleotide sequence represented by
- is, unlike conventional primers, capable of selectively SEQ ID NO:11; amplifying a broad area covering from the second membraneother domains of G protein coupled receptor proteins. spanning domain to the seventh membrane-spanning domain or
- 30 combination of Among the DNA primers of the present invention, the
- 35 of a DNA primer having a nucleotide sequence represented by SEQ nucleotide sequence represented by SEQ ID NO: 14 and a DNA sequence represented by SEQ ID NO: 7, a DNA primer having a represented by SEQ ID NO: 6, a DNA primer having a nucleotide by SEQ ID NO: 5, a DNA primer having a nucleotide sequence ID NO: 3, a DNA primer having a nucleotide sequence represented at least one DNA primer selected from the group consisting

primer having a nucleotide sequence represented by SEQ ID NO:

② a DNA primer having a nucleotide sequence represented by SEQ ID NO: 11;

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spanning domain to the seventh membrane-spanning domain or amplifying a broad area covering from the third membraneis, unlike conventional primers, capable of selectively other domains of G protein coupled receptor proteins.

protein coupled receptor proteins and the homology at the amino acid level or the nucleic acid level between G protein coupled Therefore, the protein hydrophobicity plotting of G hydrophobicity plotting and homology both serve as standards coupled receptor protein] can now be more clearly calculated. encoding part or all of the amino acid seguence of G protein receptor proteins and other similar receptor proteins (said thereof) obtained according to the present invention is(are) for determining whether or not RNA or DNA (or fragment(s)

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Among the DNA primers of the present invention, the combination of

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of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 3, a DNA primer having a nucleotide sequence represented at least one DNA primer selected from the group consisting represented by SEQ ID NO: 6, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 7, a DNA primer having a by SEQ ID NO: 5, a DNA primer having a nucleotide sequence nucleotide sequence represented by SEQ ID NO: 14 and a DNA Primer having a nucleotide sequence represented by SEQ ID NO: 18; with

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of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 2, a DNA primer having a nucleotide seguence represented a DNA primer having a nucleotide sequence represented by SEQ ID at least one DNA primer selected from the group consisting nucleotide sequence represented by SEQ ID NO: 15, a DNA primer represented by SEQ ID NO: 8, a DNA primer having a nucleotide having a nucleotide sequence represented by SEQ IS NO: 17 and seguence represented by SEQ ID NO: 9, a DNA primer having a by SEQ ID NO: 4, a DNA primer having a nucleotide sequence

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membrane-spanning domain to the sixth membrane-spanning domain amplifying DNA coding for G protein coupled receptor proteins though it has not been obtained through the conventional DNA moreover, it is capable of more selectively and efficiently is capable of amplifying the areas covering from the third thereof at once like the conventional DNA primers and,

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Moreover, among the DNA primers of the present invention, the combination of

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at least one DNA primer selected from DNA primers having a nucleotide sequence of SEQ ID NO: 1 and DNA primers having a nucleotide sequence of SEQ ID NO: 12; with

membrane-spanning domain to the third membrane-spanning domain ② a DNA primer having a nucleotide sequence represented by is capable of amplifying the areas covering from the first SEQ ID NO: 13;

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thereof at once.

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(c) the amplified DNA (or fragment(s) thereof) coding for the coding for the amino acid sequence of from the first membraneamino acid sequence of from the third membrane-spanning domain Then (a) the amplified DNA (or fragment(s) thereof) thereof) coding for the amino acid sequence of from the third membrane-spanning domain of G protein coupled receptor proté spanning domain to the sixth membrane-spanning domain of G protein coupled receptor protein, (b) the amplified DNA (or fragment(s) thereof) coding for the amino acid sequence of to the sixth membrane-spanning domain of G protein coupled membrane-spanning domain to the seventh membrane-spanning receptor protein, (d) the amplified DNA (or fragment(s) from the first membrane-spanning domain to the seventh

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DNA (or fragment(s) thereof) coding for the amino acid seguence amino acid sequence of from the second membrane-spanning domain membrane-spanning domain of G protein coupled receptor protein, (f) the amplified DNA (or fragment(s) thereof) coding for the of from the second membrane-spanning domain to the sixth

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domain of G protein coupled receptor protein, (e) the amplified

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used as a probe(s) to screen for full-length DNA which DNA libraries according to methods known per se by those of completely encodes G protein coupled receptor proteins from other domains of G protein coupled receptor protein may be of G protein coupled receptor protein or (h) the amplified DNA skill in the art or methods similar thereto. (or fragment(s) thereof) coding for the amino acid sequence of membrane-spanning domain to the third membrane-spanning domain thereof) coding for the amino acid sequence of from the first receptor protein, (g) the amplified DNA (or fragment(s) to the seventh membrane-spanning domain of G protein coupled

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herein refers to a DNA library or DNA libraries including all libraries. The term "DNA library" or "DNA libraries" as used of those libraries. include any of genome DNA libraries, cDNA libraries and RNA The DNA libraries used in the present invention

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containing DNA (or fragment(s) thereof) coding for receptor proteins, which comprise employing the DNA of the present protein coupled receptor protein from the DNA library methods for target DNA (or fragment(s) thereof) coding for G invention as a DNA primer for the PCR. The present invention further provides screening

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from DNA libraries which comprises the steps of an amino acid sequence of G protein coupled receptor protein a method for cloning full-length DNA which completely encodes (i) using the DNA of the present invention as a DNA primer One preferred embodiment of the present invention is

30 DNA primer with the DNA library to amplify and select (i.e. (ii) carrying out PCR in the presence of a mixture of said

coding for the amino acid sequence of from the third membranedomain of G protein coupled receptor protein, a DNA fragment first membrane-spanning domain to the seventh membrane-spanning a DNA fragment coding for the amino acid sequence of from the membrane-spanning domain of G protein coupled receptor protein, of from the first membrane-spanning domain to the sixth screen for) a DNA fragment coding for the amino acid sequence

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15 10 S a DNA fragment coding for other domains of G protein coupled according to cloning methods known per se by those of skill spanning domain of G protein coupled receptor protein or sequence of from the second membrane-spanning domain to the acid sequence of from the second membrane-spanning domain to coupled receptor protein, a DNA fragment coding for the amino the DNA fragment obtained in the above step (ii). in the art or methods similar thereto by using, as a probe, receptor protein; and from the first membrane-spanning domain to the third membraneprotein, a DNA fragment coding for the amino acid sequence of seventh membrane-spanning domain of G protein coupled receptor receptor protein, a DNA fragment coding for the amino acid the sixth membrane-spanning domain of G protein coupled domain to the seventh membrane-spanning domain of G protein the amino acid seguence of from the third membrane-spanning protein coupled receptor protein, a DNA fragment coding for (iii) cloning said full-length DNA from the DNA library spanning domain to the sixth membrane-spanning domain of G

carrying out a polymerase chain reaction in the presence of receptor proteins from DNA libraries, which comprises is a screening method of DNA coding for G protein coupled Preferably, an embodiment of the present invention

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25 the DNA library,

a nucleotide sequence represented by SEQ ID NO: 6, DNA primers of DNA primers having a nucleotide sequence represented by a nucleotide sequence represented by SEQ ID NO: 18 and SEQ ID NO: 10, DNA primers having a nucleotide sequence DNA primers having a nucleotide sequence represented by having a nucleotide sequence represented by SEQ ID NO: 7, sequence represented by SEQ ID NO: 5, DNA primers having represented by SEQ ID NO: 3, DNA primers having a nucleotide SEQ ID NO: 1, DNA primers having a nucleotide seguence sequence represented by SEQ ID NO: 16 and DNA primers having represented by SEQ ID NO: 14, DNA primers having a nucleotide at least one DNA primer selected from the group consisting

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(3) at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 11, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19 to selectively amplify template DNA coding for G protein coupled receptor protein contained in the DNA library.

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More preferably, embodiments of the present invention

include:

(1) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the first transmembrane domain to the sixth transmembrane domain of G protein coupled receptor protein or other domains thereof) from a DNA library, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

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① the DNA library,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 12 and

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© at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19

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to selectively amplify the DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the first transmembrane domain to the sixth transmembrane domain of G protein coupled receptor protein or other domains thereof) contained in the DNA library;

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(2) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the first transmembrane domain.

to the seventh transmembrane domain of G protein coupled receptor protein or other domains thereof) from a DNA library, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

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① the DNA library,

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② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 12 and

③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 11

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to selectively amplify the DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the first transmembrane domain

25 to the seventh transmembrane domain of G protein coupled receptor protein or other domains thereof) contained in the DNA library; sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the second transmembrane domain to the sixth transmembrane domain of G protein coupled receptor protein or other domains thereof) from a DNA library, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

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35 ① the DNA library,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by

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SEQ ID NO: 10 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 16 and

- ② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers.
- having a nucleotide sequence represented by SEQ ID NO: 9, DNA primer having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19
- to selectively amplify the DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the second transmembrane domain to the sixth transmembrane domain of G protein coupled receptor protein or other domains thereof) contained in the DNA library;

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- (4) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the second transmembrane domain to the seventh transmembrane domain of G protein coupled receptor protein or other domains thereof) from a DNA library, which comprises carrying out a polymerase chain reaction in the presence of a mixture of
- W the DNA library,
- ② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 10 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 16 and

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- ② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 11
- to selectively amplify the DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the second transmembrane domain to the seventh transmembrane domain of G protein coupled

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receptor protein or other domains thereof) contained in the DNA library;

- (5) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the third transmembrane domain to the sixth transmembrane domain of G protein coupled receptor protein or other domains thereof) from a DNA library, which comprises carrying out a polymerase chain reaction in the presence of a mixture of
- 10 ① the DNA library,
- ② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 3, DNA primers having a nucleotide sequence represented by SEQ ID NO: 5, DNA primers having a nucleotide sequence represented by SEQ ID NO: 6, DNA primers having a nucleotide sequence represented by SEQ ID NO: 7, DNA primers having a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 18 and

- 20 ② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15,
- a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19
- 30 to selectively amplify the DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the third transmembrane domain to the sixth transmembrane domain of G protein coupled receptor protein or other domains thereof) contained in the DNA library;
- (6) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein and the like

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(e.g. the regions spanning from the third transmembrane domain to the seventh transmembrane domain of G protein coupled receptor protein or other domains thereof) from a DNA library, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

the DNA library,

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- at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 3, DNA primers having a nucleotide sequence represented by SEQ ID NO: 5, DNA primers having a nucleotide
  - represented by SEQ ID NO: 5, DNA primers having a nucleotide sequence represented by SEQ ID NO: 6, DNA primers having a nucleotide sequence represented by SEQ ID NO: 7, DNA primers having a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers having a nucleotide sequence represented by
    - 15 SEQ ID NO: 18 and
- ③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 11
- to selectively amplify the DNA coding for the amino acid
  sequence of G protein coupled receptor protein and the like
  (e.g. the regions spanning from the third transmembrane
  domain to the seventh transmembrane domain of G protein coupled
  receptor protein or other domains thereof) contained in
  the DNA library; and
- sequence of G protein coupled receptor protein and the like

  (e.g. the regions spanning from the first transmembrane
  domain to the third transmembrane domain of G protein coupled
  receptor protein or other domains thereof) from a DNA library,
  which comprises carrying out a polymerase chain reaction in the
  presence of a mixture of
- the DNA library,
- ② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 12 and

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at least one DNA primer selected from the group consisting

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of DNA primers having a nucleotide sequence represented by SEQ ID NO: 13

to selectively amplify the DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the first transmembrane domain to the third transmembrane domain of G protein coupled

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receptor protein or other domains thereof) contained in the DNA library.

Particularly preferably, embodiments of the present

invention include:

(8) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein from a DNA library, which comprises carrying out a polymerase chain

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15 ( the DNA library,

reaction in the presence of a mixture of

- a DNA primer having a nucleotide sequence represented by SEQ ID NO: 1 and
- ② a DNA primer having a nucleotide sequence represented by SEQ ID NO: 2
  - 20 to selectively amplify the DNA coding for the amino acid sequence of G protein coupled receptor protein contained in the DNA library;
- (9) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein from a DNA library, which comprises carrying out a polymerase chain
- 11 library, which comprises carrying out a polymerase chain reaction in the presence of a mixture of
- ) the DNA library,
- ② a DNA primer having a nucleotide sequence represented by SEQ ID NO: 3 and
- 30 ③ a DNA primer having a nucleotide sequence represented by SEQ ID NO: 4 to selectively amplify the DNA coding for the amino acid
  - to selectively amplify the DNA coding for the amino acid sequence of G protein coupled receptor protein contained in the DNA library;
    - (10) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein from a DNA library, which comprises carrying out a polymerase chain

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reaction in the presence of a mixture of

- the DNA library,
- SEQ ID NO: 6 and a DNA primer having a nucleotide sequence represented by
- S Θ SEQ ID NO: 8 a DNA primer having a nucleotide sequence represented by
- (11) a screening method of DNA coding for the amino acid the DNA library; and sequence of G protein coupled receptor protein contained in to selectively amplify the DNA coding for the amino acid reaction in the presence of a mixture of library, which comprises carrying out a polymerase chain sequence of G protein coupled receptor protein from a DNA
- the DNA library,
- 15 SEQ ID NO: 10 and a DNA primer having a nucleotide sequence represented by
- a DNA primer having a nucleotide sequence represented by
- 20 sequence of G protein coupled receptor protein contained in to selectively amplify the DNA coding for the amino acid SEQ ID NO: 11

the DNA library. The cloned DNAs can be analyzed, usually by

restriction enzyme analysis and/or sequencing. coding for unknown (novel) G protein coupled receptor coupled receptor proteins and RNA, DNA or fragments thereof the present invention is employed may include RNA, DNA or and the screening by the PCR techniques wherein the DNA of for G protein coupled receptor protein in the amplification fragments thereof coding for known (or prior art) G protein Target RNA or DNA (or fragment(s) thereof) coding

proteins. include novel nucleotide sequences and even known nucleotide These target RNA or DNA (or fragment(s) thereof) may

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protein, said RNA or DNA (or fragment(s)) being derived from (or fragment(s)) coding for a G protein coupled receptor Examples of such nucleotide sequences are RNA or DNA

G protein coupled receptor proteins or DNA fragments (or

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S rats, cats, dogs, swines, cattle, horses, monkeys, human lung, adrenal gland, etc.) of vertebrate animals (e.g. mice, all cells and tissues (e.g. pituitary gland, brain, pancreas, drosophilae, silkworms, Barathra brassicae, etc.), plants beings, etc.), insects or other invertebrate animals (e.g. lines derived therefrom, etc. (e.g. rice plant, wheat, tomato, etc.) and cultured cell

5 15 20 oxytocin, VIP (vasoactive intestinal and related peptide), melatonin, neuropeptide Y, opioid, purine, vasopressin, bombesin, canavinoid, cholecystokinin, glutamine, serotonin, receptor proteins such as receptor proteins to angiotensin, RNA or DNA (or fragment(s)) coding for G protein coupled pancreastatin, prostaglandin, thromboxane, adenosine, (calcitonin gene related peptide), adrenomedullin, leukotriene, somatostatin, dopamine, motilin, amylin, bradykinin, CGRP histamine, neurotensin, TRH, pancreatic polypeptide, galanin, MIP1a , MIP-1eta , RANTES, etc.), endothelin, enterogastrin, NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, adrenaline, a - and  $\beta$  -chemokine (IL-8, GROa , GRO $\beta$  , GRO $\tau$  , family members thereof, etc. Specific examples of the nucleotide sequences are

35 above-mentioned tissues and cells. More specifically, the template may include any DNA so far as it is derived from the particularly suitable. Vectors to be used in the DNA library libraries derived from human tissues and cells are and cDNA libraries derived from the tissues and cells. cDNA genome DNA libraries, cDNA derived from the tissues and cells template DNA (or DNA fragment) includes any of genome DNA, present invention, the DNA (or DNA fragment) acting as a may include any of bacteriophages, plasmids, cosmids, phagimids, etc. It is also possible to directly amplify the fractions prepared from the tissues and cells. The DNA which template DNA (or DNA fragment) by reverse transcriptase is to be a template may be either DNA completely coding for polymerase chain reaction (RT-PCR) techniques using mRNA In the PCR amplification using the DNA of the

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segments) thereof.

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thereof) contained in the used DNA library. More specifically, Preferably, the RNA or DNA (or fragment(s) thereof) coupled receptor protein coding DNA wherein said method uses receptor, melatonin receptor, neuropeptide Y receptor, opioid coupled receptor protein-encoding RNA or DNA (or fragment(s) (hereinafter, may be often abbreviated as just "DNA") coding adenosine receptor, adrenaline receptor, a - and eta -chemokine for G protein coupled receptor proteins such as angiotensin motilin receptor, amylin receptor, bradykinin receptor, CGRP adrenomedullin receptor, leukotriene receptor, pancreastatin Peptide receptor), somatostatin receptor, dopamine receptor, it is an RNA or DNA (or RNA fragment(s) or DNA fragment(s) the DNA according to the present invention is a G protein NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, receptor, purine receptor, vasopressin receptor, oxytocin receptor, VIP receptor (vasoactive intestinal and related obtained via the instant screening method for G protein cholecystokinin receptor, glutamine receptor, serotonin receptor, TRH receptor, pancreatic polypeptide receptor, receptor, prostaglandin receptor, thromboxane receptor, enterogastrin receptor, histamine receptor, neurotensin receptor (calcitonin gene related peptide receptor), galanin receptor, their family member receptors, etc. MIPla , MIP-1 $\beta$  , RANTES, etc.), endothelin receptor, receptor, bombesin receptor, canavinoid receptor, receptor (receptors to IL-8, GRO a , GROeta , GROeta ,

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Present invention is the DNA fragment which partially codes for a G protein coupled receptor protein, it is possible to isolate DNA completely encoding said G protein coupled receptor protein When the DNA obtained by the screening method of the from a suitable DNA library according to cloning techniques known per se by using said DNA fragment as a probe.

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Means for cloning the DNA completely encoding G amplification employing a synthetic DNA primer having the Partial nucleotide sequence of the DNA fragment partially protein coupled receptor proteins may include a PCR

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fragments. The hybridization may be conducted, for example, conducted according to the manners described in the  $\operatorname{protocol}_q$ selection of the target DNA via a hybridization with DNA or synthetic DNA having part or all of the region of said DNA When the commercially available library is used, it may be coding for the G protein coupled receptor protein and a J. Sambrook et al., Cold Spring Harbor Lab. Press, 1989. by the methods described in Molecular Cloning, 2nd ed.; attached thereto.

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ATG at the 5'-terminal as the translation initiation codon and translation termination codons may be added using a suitable by northern blottings using said DNA as a probe. It is also protein DNA) may be used, depending upon its object, either after ligating with a linker if desired. Said DNA may have termination codon. These translation initiation codons and DNA having the entire coding region of the receptor protein possible to express target receptor proteins by introducing into animal cells after binding with a suitable promoter. receptor protein (full-length G protein coupled receptor as it is or after digesting with a restriction enzyme or determine said receptor protein-expressing tissues/cells The DNA completely encoding G protein coupled synthetic DNA adaptor. In addition, it is possible to TAA, TGA or TAG at the 3' terminal as the translation

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coupled receptor proteins such as angiotensin receptor protein, the present invention is a G protein coupled receptor protein protein according to the present invention includes G protein invention. More specifically, the G protein coupled receptor cholecystokinin receptor protein, glutamine receptor protein, The G protein coupled receptor protein according encoded by the G protein coupled receptor protein-encoding oxytocin receptor protein, VIP receptor protein (vasoactive neuropeptide Y receptor protein, opioid receptor protein, bombesin receptor protein, canavinoid receptor protein, serotonin receptor protein, melatonin receptor protein, purine receptor protein, vasopressin receptor protein, DNA obtained by the screening method of the present

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protein), adrenomedullin receptor protein, leukotriene receptor protein, amylin receptor protein, bradykinin receptor protein, CGRP receptor protein (calcitonin gene related peptide receptor receptor protein, dopamine receptor protein, motilin receptor intestinal and related peptide receptor protein), somatostatin

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receptor protein, family members thereof, etc. protein, pancreatic polypeptide receptor protein, galanin MCP-3, I-309, MIP1 $\alpha$  , MIP-1 $\beta$  , RANTES, etc.), endothelin protein, adrenaline receptor protein, a - and eta -chemokine protein, thromboxane receptor protein, adenosine receptor protein, pancreastatin receptor protein, prostaglandin receptor receptor protein, neurotensin receptor protein, TRH receptor receptor protein, enterogastrin receptor protein, histamine GRO $\beta$  , GRO $\gamma$  , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, receptor protein (receptor protein responsive to IL-8, GROa ,

recognized, cloned, produced, isolated or characterized. derivatives or analogues thereof, and salts thereof may be derived from the G protein coupled receptor protein, modified coupled receptors proteins, peptide segments or fragments According to the present invention, novel G protein

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guinea pig, rat, mouse, swine, sheep, cattle, monkey, human duct, blood vessel, heart, etc.) of warm-blooded animals (e.g. SEQ ID NO: 24, an amino acid sequence represented by SEQ ID the group consisting of an amino acid sequence represented by as long as they comprise an amino acid sequence selected from cholecyst, bone marrow, adrenal, skin, muscle, lung, digestive pancreas, brain, kidney, liver, gonad, thyroid gland, represented by SEQ ID NO: 35, an amino acid sequence sequence represented by SEQ ID NO: 34, an amino acid sequence acid sequence represented by SEQ ID NO: 28, an amino acid an amino acid sequence represented by SEQ ID NO: 27, an amino NO: 25, an amino acid sequence represented by SEQ ID NO: 26, beings, rabbit, cat, dog, horse, etc.), and any of proteins derived from all cells and tissues (e.g. pituitary gland, represented by SEQ ID NO: 38, an amino acid sequence represented by SEQ ID NO: 39, an amino acid seguence These G protein coupled receptor proteins are those

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34, SEQ ID NO: 35, SEQ ID NO: 38, SEQ ID NO: 39, and/or SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: the amino acid sequence represented by SEQ ID NO: 24, SEQ ID represented by SEQ ID NO: 56, and substantial equivalents to

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15 10 20 30 25 35 sequence represented by SEQ ID NO: 24, an amino acid sequence sequence selected from the group consisting of an amino acid and any of proteins as long as they comprise an amino acid sheep, cattle, monkey, human beings, cat, dog, horse, etc.), warm-blooded animals (e.g. guinea pig, rat, mouse, swine, muscle, lung, digestive duct, blood vessel, heart, etc.) of gonad, thyroid gland, cholecyst, bone marrow, adrenal, skin, tissues (e.g. pituitary gland, pancreas, brain, kidney, liver, coupled receptor proteins are those derived from all cells and represented by SEQ ID NO: 26, an amino acid sequence represented by SEQ ID NO: 25, an amino acid sequence NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, or SEQ ID NO: 28. the amino acid sequence represented by SEQ ID NO: 24, SEQ ID represented by SEQ ID NO: 28, and substantial equivalents to represented by SEQ ID NO: 27, an amino acid sequence amino acid sequence represented by SEQ ID NO: 25, an amino acid amino acid seguence thereof is about 90% to 99.9% homologous sequence represented by SEQ ID NO: 28, proteins wherein the sequence represented by SEQ ID NO: 27 and an amino acid amino acid sequence represented by SEQ ID NO: 26, an amino acid NO: 24, an amino acid sequence represented by SEQ ID NO: 25, an consisting of an amino acid sequence represented by SEQ ID having an amino acid sequence selected from the group These G protein coupled receptor proteins may include proteins substantially equivalent to the protein having an amino acid represented by SEQ ID NO: 28 and the activity thereof is represented by SEQ ID NO: 27 or an amino acid sequence sequence represented by SEQ ID NO: 26, an amino acid sequence to an amino acid sequence represented by SEQ ID NO: 24, an represented by SEQ ID NO: 25, an amino acid sequence sequence represented by SEQ ID NO: 24, an amino acid sequence In one embodiment of the present invention, G protein

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represented by SEQ ID NO: 26, an amino acid sequence represented by SEQ ID NO: 27 or an amino acid sequence represented by SEQ ID NO: 28 and the like. The substantially equivalent activity may include ligand binding activity, signal information transmitting, etc. The term "substantially equivalent" or "substantial equivalent" means that the nature of the ligand binding activity and the like is equivalent. Therefore, it is allowable that even differences among grades such as ligand binding affinity grades and ligand binding activity grades and quantitative factors such as molecular weights of receptor proteins are present.

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pancreas-derived G protein coupled receptor proteins comprising pancreas-derived G protein coupled receptor proteins comprising Examples of the human pituitary gland-derived G protein coupled from the group consisting of an amino acid sequence represented by SEQ ID NO: 24, and an amino acid sequence represented by SEQ receptor proteins comprising an amino acid sequence represented ID NO: 26, SEQ ID NO: 27 or SEQ ID NO: 28, proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are ID NO: 25, are human pituitary gland-derived G protein coupled preferably from 2 to 10 amino acid residues) are deleted from added to the amino acid sequence of SEQ ID NO: 24, SEQ ID NO: gland-derived G protein coupled receptor proteins comprising the amino acid sequence of SEQ ID NO: 24, SEQ ID NO: 25, SEQ an amino acid sequence selected from the group consisting of proteins may include proteins wherein one or more amino acid In another embodiment of the present invention, G an amino acid seguence represented by SEQ ID NO: 24, and/or receptor protein comprising an amino acid sequence selected residues (preferably from 2 to 30 amino acid residues, more 25, SEQ ID NO: 26, SEQ ID NO: 27 or SEQ ID NO: 28, proteins an amino acid sequence represented by SEQ ID NO: 25, mouse an amino acid sequence represented by SEQ ID NO: 27, mouse protein coupled receptor proteins include human pituitary an amino acid sequence represented by SEQ ID NO: 28, etc. by SEQ ID NO: 24, etc. These G protein coupled receptor

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wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27 or SEQ ID NO: 28, are substituted with one or more amino acid residues, etc.

neart, thymus, spleen, leukocyte, etc.) of warm-blooded animals proteins having an amino acid sequence selected from the group that the nature of the ligand binding activity and the like is among grades such as ligand binding affinity grades and ligand G protein coupled receptor proteins include those derived from NO: 35. These G protein coupled receptor proteins may include In yet another embodiment of the present invention, equivalent. Therefore, it is allowable that even differences 'substantially equivalent" or "substantial equivalent" means substantially equivalent activity may include ligand binding Examples of the G protein coupled receptor protein are human substantially equivalent to the protein having an amino acid gland, pancreas, brain, kidney, liver, gonad, thyroid gland, NO: 35, proteins wherein the amino acid sequence thereof is cholecyst, bone marrow, lung, digestive duct, blood vessel, consisting of an amino acid sequence represented by SEQ ID consisting of an amino acid sequence represented by SEQ ID NO: 34 and/or an amino acid sequence represented by SEQ ID sequence represented by SEQ ID NO: 34 and/or an amino acid NO: 34 or/and an amino acid sequence represented by SEQ ID epresented by SEQ ID NO: 34 or/and an amino acid seguence activity, signal information transmitting, etc. The term all cells and tissues (e.g. amygdaloid nucleus, pituitary sequence represented by SEQ ID NO: 35, and the like. The (e.g. guinea pig, rat, mouse, pig, sheep, cattle, monkey, represented by SEQ ID NO: 35 and the activity thereof is numan beings, etc.), and any of proteins as long as they binding activity grades and quantitative factors such as about 90% to 99.9% homologous to an amino acid sequence comprise an amino acid sequence selected from the group molecular weights of receptor proteins are present.

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amygdaloid nucleus-derived G protein coupled receptor proteins

amino acid residues) are deleted from the amino acid sequence or more amino acid residues, etc. added to the amino acid sequence of SEQ ID NO: 34 or SEQ ID NO: amino acid residues (preferably from 2 to 30 amino acid of SEQ ID NO: 34 or SEQ ID NO: 35, proteins wherein one or more proteins wherein one or more amino acid residues (preferably SEQ ID NO: 34 or SEQ ID NO: 35, are substituted with one from 2 to 10 amino acid residues) in the amino acid seguence of (preferably from 2 to 30 amino acid residues, more preferably 35, proteins wherein one or more amino acid residues residues, more preferably from 2 to 10 amino acid residues) are from 2 to 30 amino acid residues, more preferably from 2 to 10 34 and/or an amino acid sequence represented by SEQ ID NO: 35, consisting of an amino acid sequence represented by SEQ ID NO: having an amino acid sequence selected from the group These G protein coupled receptor proteins may include

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or substantial equivalents to the amino acid sequence represented by SEQ ID NO: 39, or substantial equivalents to represented by SEQ ID NO: 38, preferably an amino acid sequence vessel, heart, thymus, leukocyte, etc.) of warm-blooded animals gland, cholecyst, bone marrow, lung, digestive duct, blood comprise an amino acid sequence represented by SEQ ID NO: 38, human beings, etc.), and any of proteins as long as they (e.g. guinea pig, rat, mouse, swine, sheep, cattle, monkey, body, pancreas, brain, kidney, liver, gonad, thyroid from all cells and tissues (e.g. amygdaloid nucleus, pituitary these G protein coupled receptor proteins are those derived In still another embodiment of the present invention,

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These G protein coupled receptor proteins are preferably represented by SEQ ID NO: 38 and the like. equivalent to the protein having an amino acid sequence SEQ ID NO: 38 and the activity thereof is substantially proteins wherein the amino acid sequence thereof is about 90% having an amino acid seguence represented by SEQ ID NO: 38, These G protein coupled receptor proteins may include proteins the amino acid sequence represented by SEQ ID NO: 39. to 99.9% homologous to an amino acid sequence represented by

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10 S activity grades and quantitative factors such as molecular such as ligand binding affinity grades and ligand binding Therefore, it is allowable that even differences among grades of the ligand binding activity and the like is equivalent. equivalent" or "substantial equivalent" means that the nature signal information transmitting, etc. The term "substantially equivalent activity may include ligand binding activity, sequence represented by SEQ ID NO: 39, etc. The substantially substantially equivalent to the protein having an amino acid represented by SEQ ID NO: 39 and the activity thereof is about 90% to 99.9% homologous to an amino acid sequence NO: 39, proteins wherein the amino acid sequence thereof proteins having an amino acid sequence represented by SEQ sizes or weights of receptor proteins are present.

encoded by pMAH2-17) is a novel purinoceptor subtype which is invention (e.g., SEQ ID NO: 38 and SEQ ID NO: 39, or proteins clearly distinct from prior art purinoceptors. eta -cell strain, MIN6-derived receptor protein of the present It is suggested by data that the mouse pancreatic

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20 မ 25 35 acid residues, more preferably from 2 to 10 amino acid one or more amino acid residues (preferably from 2 to 30 amino more preferably from 2 to 10 amino acid residues) are deleted acid residues (preferably from 2 to 30 amino acid residues, by SEQ ID NO: 38, mouse pancreatic  $\beta$  -cell line, MIN6, derived receptor proteins comprising an amino acid sequence represented pancreatic eta -cell line, MIN6, derived G protein coupled invention, G protein coupled receptor proteins include mouse 38, proteins wherein one or more amino acid residues residues) are added to the amino acid sequence of SEQ ID from the amino acid sequence of SEQ ID NO: 38, proteins wherein proteins include mouse pancreatic eta -cell line, MIN6, derived G 38, etc. Further preferably these G protein coupled receptor amino acid residues in the amino acid sequence of SEQ ID NO: from 2 to 10 amino acid residues) are substituted with other (preferably from 2 to 30 amino acid residues, more preferably protein coupled receptor proteins wherein one or more amino In another more specific embodiment of the present

line, MIN6, derived G protein coupled receptor proteins wherein sequence represented by SEQ ID NO: 39, mouse pancreatic eta -cell acid sequence of SEQ ID NO: 39 are substituted with other amino more preferably from 2 to 10 amino acid residues) in the amino one or more amino acid residues (preferably from 2 to 30 amino (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 39, proteins wherein one or more amino residues) are deleted from the amino acid sequence of SEQ ID acid residues (preferably from 2 to 30 amino acid residues, protein coupled receptor proteins comprising an amino acid NO: 39, proteins wherein one or more amino acid residues acid residues, more preferably from 2 to 10 amino acid acid residues, etc.

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differences among grades such as ligand binding affinity grades In still another embodiment of the present invention, NO: 56. These G protein coupled receptor proteins may include blood vessel, heart, thymus, leukocyte, etc.) of human beings, equivalents to the amino acid sequence represented by SEQ ID proteins having an amino acid sequence represented by SEQ ID from all cells and tissues (e.g. placenta, gonad, amygdaloid thyroid gland, cholecyst, bone marrow, lung, digestive duct, binding activity, signal information transmitting, etc. The means that the nature of the ligand binding activity and the substantially equivalent to the protein having an amino acid these G protein coupled receptor proteins are those derived NO: 56, proteins wherein the amino acid sequence thereof is and ligand binding activity grades and guantitative factors term "substantially equivalent" or "substantial equivalent" and any of proteins as long as they comprise an amino acid like is equivalent. Therefore, it is allowable that even represented by SEQ ID NO: 56 and the activity thereof is nucleus, pituitary body, pancreas, brain, kidney, liver, The substantially equivalent activity may include ligand about 90% to 99.9% homologous to an amino acid seguence sequence represented by SEQ ID NO: 56, or substantial sequence represented by SEQ ID NO: 56 and the like.

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such as molecular sizes or weights of receptor proteins are

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from 2 to 10 amino acid residues) in the amino acid seguence of (preferably from 2 to 30 amino acid residues, more preferably (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 56, proteins wherein one or more In another more specific embodiment of the present residues) are added to the amino acid sequence of SEQ ID NO: protein coupled receptor proteins comprising an amino acid receptor proteins wherein one or more amino acid residues invention, G protein coupled receptor proteins include G sequence represented by SEQ ID NO: 56, G protein coupled scid residues, more preferably from 2 to 10 amino acid 56, proteins wherein one or more amino acid residues amino acid residues (preferably from 2 to 30 amino SEQ ID NO: 56, are substituted with other amino acid residues, etc.

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etc.) in the G protein coupled receptor proteins of the present A portion of the amino acid sequence may be modified (e.g. addition, deletion, substitution with other amino acids, invention.

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Furthermore, the G protein coupled receptor proteins of the present invention includes those wherein N-terminal Met pyroglutaminated, those wherein the intramolecular side chain conjugated proteins such as so-called "glycoproteins" wherein is protected with a protecting group (e.g.,  $c_{1-6}$  acyl group of amino acids is protected with a suitable protecting group such as formyl, acetyl, etc.), those wherein the N-terminal (e.g.,  $c_{1-6}$  acyl group such as formyl, acetyl, etc.), side of Glu is cleaved in vivo to make said Glu saccharide chains are bonded, etc.

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phosphoric acid, hydrobromic acid, sulfuric acid, etc.), salts The salt of said G protein coupled receptor protein of the present invention includes preferably physiologically acceptable acid addition salts. Examples of such salts are salts thereof with inorganic acids (e.g. hydrochloric acid,

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acid, methanesulfonic acid, benzenesulfonic acid, etc.), etc. tartaric acid, citric acid, malic acid, oxalic acid, benzoic propionic acid, fumaric acid, maleic acid, succinic acid, thereof with organic acids (e.g. acetic acid, formic acid,

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or cells of warm-blooded animals by purifying methods which are of the present invention may be manufactured from the tissues the peptide synthesis as described herein below. or its salt of the present invention may be manufactured by protein coupled receptor protein encoding DNA . The protein (or transfectant) (as described herein below) containing G thereto or may be manufactured by culturing the transformant known per se by those skilled in the art or methods similar The G protein coupled receptor protein or its salt

partial peptide of said G protein coupled receptor protein) may include, for example, the site which is exposed outside cell membranes, among the G protein coupled receptor protein analysis on the G protein coupled receptor protein a region which is analyzed as an extracellular area molecule. Examples of the fragment are peptides containing 44, 47, 50, 53, 57, 58, 59, 64, 70, 74, and 78. represented by any of Figures 24, 25, 28, 31, 32, 36, 38, 41, (hydrophilic region or site) in a hydrophobic plotting The G protein coupled receptor protein fragment (the

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the same time will be used as well. peptide (peptide fragment) which contains plural domains at The salt of said G protein coupled receptor protein

may be used as well. Further, a peptide which separately A peptide which partly contains a hydrophobic region or site

contains each domain may be used too although the partial

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fragment (partial peptide thereof) includes preferably such salts are salts thereof with inorganic acids (e.g. physiologically acceptable acid addition salts. Examples of succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, formic acid, propionic acid, fumaric acid, maleic acid, acid, etc.), salts thereof with organic acids (e.g. acetic hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid, benzoic acid, methanesulfonic acid, benzenesulfonic

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acid, etc.), etc.

known per se by those skilled in the art or methods similar be manufactured by synthesizing methods for peptides which are partial peptide of the G protein coupled receptor protein) may proteins by a suitable peptidase. Methods of synthesizing thereto or by cleaving (digesting) G protein coupled receptor or amino acids which can construct the protein of the present peptide may be any of a solid phase synthesis and a liquid when the product has a protective group, said protective phase synthesis. Thus, a partial peptide (peptide fragment) and for detachment of protective groups include the following manufactured. Examples of the known methods for condensation group is detached whereupon a desired peptide can be invention is condensed with the residual part thereof and, The G protein coupled receptor protein fragment (the

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M. Bodanszky and M. A. Ondetti: Peptide Synthesis, Interscience Publishers, New York (1966).

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Schroeder and Luebke: The Peptide, Academic Press, New

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York, 1965.

Nobuo Izumiya et al.: Fundamentals and Experiments of the Peptide Synthesis, Maruzen KK, Japan (1975). Haruaki Yajima and Shumpei Sakakibara: "Seikagaku Jikken

p.205 (1977), Japan. Koza 1" (Experiments of Biochemistry, Part 1), "Tanpakusitu No Kagaku IV" (Chemistry of Protein, IV),

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Ø Haruaki Yajima (ed): Development of Pharmaceuticals Shoten, Japan. (Second Series), Vol. 14, Peptide Synthesis, Hirokawa

such as salting-out, extraction with solvents, distillation, When the protein obtained as such is a free compound, it may be column chromatography, liquid chromatography, electrophoresis, protein of the present invention can be purified and isolated. recrystallization, etc. are optionally combined so that the After the reaction, conventional purifying techniques

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converted to a suitable salt by known methods while, when it is obtained as a salt, the salt may be converted to a free compound or other salt compounds by known methods.

culturing the transformant (transfectant) containing the DNA Furthermore, the product may be manufactured by coding for said partial peptide.

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obtained by the above-mentioned screening method using the DNA peptide thereof) encoded by said DNA may, for example, be used The G protein coupled receptor protein-encoding DNA inhibits the binding of said protein coupled receptor protein protein encoded by said DNA or the peptide fragment (partial of the present invention and the G protein coupled receptor for the determination of a ligand to said G protein coupled receptor protein or for the screening of a compound which with a ligand.

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In that case, an expression system for the G protein coupled receptor protein-encoding DNA is at first constructed. Promoters used therefor may be anyone so far as it is suitable Hosts for said DNA may be any of animal cells, insect cells, Incidentally, the utilization of enhancers for expression is as a promoter for the host used for gene expression. Yeasts, Bacillus subtilis, Escherichia coli, etc. effective as well.

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while, in the case of floating cells or cell membrane fractions to express the G protein coupled receptor protein or the cell membrane fractions prepared therefrom by methods known per se by those skilled in the art or methods similar thereto may be Ligands used therefor may include any of compounds labeled by separation or filtration. Nonspecific binding with container, Then the expressing cells per se which constructed Separation of bonded or free ligands may be carried out by a Supernatants and tissue extracts which are directly labeled direct washing when cells adhered to substrates are used, by a chloramine T method or by a lactoperoxidase method. subjected to a variety of receptor binding experiments, thereof, it may be carried out by means of centrifugal a Commercially available radioisotope, etc., culture

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etc. may be estimated by addition of unlabeled ligands which are about 100 times as much concentrated relatively to the Poured labeled ligand.

binding experiment may be subjected to a discrimination of The ligand which is obtained by such a receptor agonist versus antagonist. To be more specific, a natural substance or compound which is obtained by such a screening is an agonist for said G protein coupled receptor protein and is presumed to act on the coupled receptor protein-encoding DNA selectively from tissues. having a novel pharmaceutical response (pharmaceutical effect) which is presumed to be a ligand with the G protein coupled receptor protein-expressing cell is cultured and, after that kit (e.g. kits for CAMP, diacylglycerol, cGMP, proteinkinase measured by, for example, a commercially available measuring protein coupled receptor protein or an antagonist for said G kidney, pancreas, etc. is expected. An efficient development response (pharmaceutical effect) more efficiently by referring in, for example, central nervous tissues, circulatory system, extracted. A change in the components contained therein is  $\begin{bmatrix} ^3H \end{bmatrix}$ arachidonic acid and  $\begin{bmatrix} ^3H \end{bmatrix}$ inosítol phosphate metabolites methods similar thereto. The compound or natural substance the culture supernatant liquid is collected or the cell is blotting or the like. Moreover, a development of compounds of pharmaceuticals can be proceeded by amplifying G protein tissues and cells in which said receptor is distributed. to the distribution disclosed (clarified) by a northern Accordingly, it is possible to check the pharmaceutical by methods known per se by those skilled in the art or Physiological responses such as liberation of Pura-2, A, etc.). Alternatively, it is possible to measure

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coupled receptor protein which contains an amino acid seguence The G protein coupled receptor protein-encoding DNA substantially equivalent to the amino acid sequence having of the present invention may be any coding DNA as long as it contains a nucleotide sequence coding for a G protein SEQ ID NO: 24 and/or which has an activity substantially

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a G protein coupled receptor protein which contains an amino substantially equivalent to the amino acid sequence having SEQ acid sequence substantially equivalent to the amino acid eguivalent to the amino acid sequence having SEQ ID NO: 24, an amino acid sequence substantially equivalent to the amino ID NO: 25, a G protein coupled receptor protein which contains sequence having SEQ ID NO: 25 and/or which has an activity substantially equivalent to the amino acid sequence having SEQ acid sequence having SEQ ID NO: 26 and/or which has an activity an amino acid sequence substantially equivalent to the amino ID NO: 26, a G protein coupled receptor protein which contains contains an amino acid sequence substantially equivalent to the substantially equivalent to the amino acid sequence having SEQ acid sequence having SEQ ID NO: 27 and/or which has an activity amino acid sequence having SEQ ID NO: 28 and/or which has an ID NO: 27, or a G protein coupled receptor protein which having SEQ ID NO: 28. activity substantially equivalent to the amino acid sequence

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DNA of the present invention may be any coding DNA as long as substantially equivalent to the amino acid sequence having coupled receptor protein which contains an amino acid sequence it contains a nucleotide sequence coding for a G protein or a G protein coupled receptor protein which contains an amino equivalent to the amino acid sequence having SEQ ID NO: 34, SEQ ID NO: 34 and/or which has an activity substantially sequence having SEQ ID NO: 35 and/or which has an activity acid sequence substantially equivalent to the amino acid substantially equivalent to the amino acid sequence having SEQ Still the G protein coupled receptor protein-encoding

substantially equivalent to the amino acid sequence having coupled receptor protein which contains an amino acid sequence DNA of the present invention may be any coding DNA as long as SEQ ID NO: 38 and/or which has an activity substantially it contains a nucleotide sequence coding for a G protein eguivalent to the amino acid sequence having SEQ ID NO: 38, or Yet the G.protein coupled receptor protein-encoding

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substantially equivalent to the amino acid sequence having SEQ acid sequence having SEQ ID NO: 39 and/or which has an activity an amino acid sequence substantially equivalent to the amino preferably a G protein coupled receptor protein which contains

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substantially equivalent to the amino acid sequence having coupled receptor protein which contains an amino acid sequence DNA of the present invention may be any coding DNA as long as acid sequence having SEQ ID NO: 56 and/or which has an activity equivalent to the amino acid sequence having SEQ ID NO: 56, or SEQ ID NO: 56 and/or which has an activity substantially it contains a nucleotide seguence coding for a G protein substantially equivalent to the amino acid sequence having SEQ preferably a G protein coupled receptor protein which contains an amino acid sequence substantially equivalent to the amino Yet the G protein coupled receptor protein-encoding

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20 25 and cell-derived cDNA, a human tissue and cell-derived cDNA referred to as "RT-PCR") using mRNA fractions prepared from transcriptase polymerase chain reaction (hereinafter briefly The DNA can be further amplified directly by the reverse may include bacteriophage, plasmid, cosmid, phagemid, etc. library and a synthetic DNA. The vector used for the library human genome DNA, a human genome DNA library, a human tissue tissues and cells. The DNA of the present invention may be any one of a

DNA having a nucleotide sequence represented by SEQ ID NO: 29, comprising the amino acid sequence of SEQ ID NO: 24 includes pituitary gland-derived G protein coupled receptor protein NO: 26 includes DNA having a nucleotide seguence represented by receptor protein comprising the amino acid sequence of SEQ ID for the human pituitary gland-derived G protein coupled sequence represented by SEQ ID NO: 30, etc. The DNA coding sequence of SEQ ID NO: 25 includes DNA having a nucleotide protein coupled receptor protein comprising the amino acid The DNA coding for the human pituitary gland-derived G In an embodiment, the DNA coding for the human

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derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 27 includes DNA having a nucleotide sequence represented by SEQ ID NO: 32, etc. The DNA coding for DNA having a nucleotide sequence represented by SEQ ID NO: 33, the mouse pancreas-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 28 includes SEQ ID NO: 31, etc. The DNA coding for the mouse pancreas-

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The DNA coding for the human amygdaloid nucleus-derived G NO: 34 or the amino acid sequence of SEQ ID NO: 35 includes DNA DNA having a nucleotide sequence represented by SEQ ID NO: 36, by SEQ ID NO: 40, etc. The DNA coding for the mouse pancreatic In another embodiment, the DNA coding for the human amygdaloid nucleus-derived G protein coupled receptor protein human-derived G protein coupled receptor protein comprising the DNA having a nucleotide sequence represented by SEQ ID NO: 37, receptor protein comprising the amino acid seguence of SEQ ID comprising the amino acid sequence of SEQ ID NO: 34 includes mouse pancreatic eta -cell line, MIN6-derived G Protein coupled receptor protein comprising the amino acid seguence of SEQ ID eta -cell line, MIN6-derived G protein coupled receptor protein DNA having a nucleotide sequence represented by SEQ ID NO: 41, NO: 38 includes DNA having a nucleotide sequence represented comprising the amino acid sequence of SEQ ID NO: 39 includes sequence represented by SEQ ID NO: 37, etc. The DNA coding protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 35 includes DNA having a nucleotide for the human amygdaloid nucleus-derived G protein coupled having a nucleotide sequence represented by SEQ ID NO: 36, amino acid seguence of SEQ ID NO: 56 includes DNA having a etc. Still in another embodiment, the DNA coding for the etc. Yet in another embodiment, the DNA coding for the nucleotide sequence represented by SEQ ID NO: 57, etc.

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The DNA completely coding for the G protein coupled (1) carrying out the PCR amplification using a synthetic DNA receptor protein of the present invention can be cloned by primer having a partial nucleotide sequence (nucleotide

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DNA fragment having part or all of the region encoding a human G protein coupled receptor protein or a labeled synthetic DNA used, the hybridization is carried out according to protocols suitable vector, based on the hybridization with a labeled When a DNA library commercially available in the market is disclosed in, for example, Molecular Cloning, 2nd Ed., J. The hybridization is carried out according to methods as fragment) of the G protein coupled receptor protein; or (2) effecting the selection of a DNA constructed in a Sambrook et al., Cold Spring Harbor Lab. Press, 1989. having part or all of the coding region thereof. manuals attached thereto.

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encoding DNA of the present invention can be used as it is, or a termination codon, TAA, TGA or TAG, on the 3' terminal side. digestion with a restriction enzyme or addition of a linker or adapter, etc. depending upon objects. The DNA may have These initiation and termination codons can be ligated by The cloned G protein coupled receptor proteincan be used, as desired, after modifications including an initiation codon, ATG, on the 5' terminal side and using a suitable synthetic DNA adapter.

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the target DNA fragment with the downstream site of a promoter An expression vector for G protein coupled receptor proteins can be produced by, for example, (a) cutting out a protein-encoding DNA of the present invention and (b) ligati target DNA fragment from the G protein coupled receptor in a suitable expression vector.

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PC194, etc.), plasmids derived from yeasts (e.g., pSH19, pSH15, etc.), bacteriophages such as  $\lambda$  -phage, and animal virus such Escherichia coli (e.g., pBR322, pBR325, pUC12, pUC13, etc.), plasmids derived from Bacillus subtilis (e.g., pUBll0, pTP5, The vector may include plasmids derived from as retrovirus, vaccinia virus and baculovirus.

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for expressing a gene. When the host for the transformation is According to the present invention, any promoter can be used as long as it is compatible with a host which is used E. coli, the promoters are preferably trp promoters, lac

cytomegalovirus promoters, SRa promoters, etc. An enhancer promoters are preferably SPO1 promoters, SPO2 promoters, penP When the host for the transformation is the Bacillus, the promoters, recA promoters,  $\lambda_{\rm PL}$  promoters, lpp promoters, etc. can be effectively utilized for the expression. metallothionein promoters, heat shock promoters, include SV40-derived promoters, retrovirus promoters, promoters, etc. When the host is an animal cell, the promoters preferably PHO5 promoters, PGK promoters, GAP promoters, ADH promoters, etc. When the host is an yeast, the promoters are

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coupled receptor protein. When the host is E. coli, the is the Bacillus, they may include a -amylase signal sequences signal sequences, OmpA signal sequences, etc. When the host utilizable signal sequences may include alkaline phosphatase may include insulin signal sequences, a -interferon signal signal seguences, etc. When the host is an animal cell, they they may include mating factor a signal sequences, invertase subtilisin signal sequences, etc. When the host is an yeast, sequences, antibody molecule signal sequences, etc. sequence is added to the N-terminal side of the G protein As required, furthermore, a host-compatible signal

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Vol. 60, 160 (1968)], JM103 [Nucleic Acids Research, Vol. 9, include Escherichia coli K12-DH1 [Proc. Natl. Acad. Sci. USA, etc. Examples of the Escherichia and Bacillus microorganisms Bacillus microorganisms, yeasts, insect cells, animal cells, The host may be, for example, Escherichia microorganisms, coupled receptor protein-encoding DNA of the present invention. the vector thus constructed, which carries the G protein A transformant or transfectant is produced by using

35 30 309 (1981)], JA221 [Journal of Molecular Biology, Vol. 120, 517 (1969)], C600 [Genetics, Vol. 39, 440 (1954)], etc. (1978)], HB101 [Journal of Molecular Biology, Vol. 41, 459 [Journal of Biochemistry, Vol. 95, 87 (1984)], etc. Bacillus subtilis MI114 [Gene, Vol. 24, 255 (1983)], 207-21 Examples of the Bacillus microorganism are, for example,

AH22R , NA87-11A, DKD-5D, 20B-12, etc. The insect may include The yeast may be, for example, Saccharomyces cerevisiae AH22,

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υ Chinese hamster cell line (dhfr CHO cell), mouse L cell, hamster ovary cell line (CHO cell), DHFR gene-deficient example, monkey-derived cell line, COS-7, Vero, Chinese a silkworm (Bombyx mori larva), [Maeda et al, Nature, Vol. 315, 592 (1985)] etc. The host animal cell may be, for

done using standard techniques appropriate to such cells. murine myeloma cell, human FL cell, etc. Depending on the host cell used, transformation is

10 17, 107 (1982), etc. Transformation of Bacillus microorganisms out in accordance with methods as disclosed in, for example, for example, Molecular & General Genetics, Vol. 168, 111 can be carried out in accordance with methods as disclosed in, Proc. Natl. Acad. Sci. USA, Vol. 69, 2110 (1972), Gene, Vol. Transformation of Escherichia microorganisms can be carried

15 as disclosed in, for example, Bio/Technology, 6, 47-55, The insect cells can be transformed in accordance with methods Proc. Natl. Acad. Sci. USA, Vol. 75, 1929 (1978), etc. in accordance with methods as disclosed in, for example, (1979), etc. Transformation of the yeast can be carried out

20 protein-encoding DNA are produced according to the with expression vectors containing a G protein coupled receptor as disclosed in, for example, Virology, Vol. 52, 456, 1973, 1988. The animal cells can be transformed by methods The transformants or transfectants which are transformed

25 aforementioned techniques.

may include glucose, dextrin, soluble starch, sucrose, etc. etc. necessary for growing the transformant. The carbon source medium may contains carbon sources, nitrogen sources, minerals, carried out suitably in a liquid culture medium. The culture casein, meat extracts, bean-cakes, potato extracts, etc. The nitrogen source may include organic or inorganic substances which the host is Escherichia or Bacillus microorganism can be such as ammonium salts, nitrates, corn steep liquor, peptone, Cultivation of the transformant (transfectant) in

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35 It is further allowable to add yeasts, vitamines, growthsodium dihydrogen phosphate, magnesium chloride, etc. Examples of the minerals may include calcium chloride,

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promoting factors, etc. It is desired that the culture medium is pH from about 5 to about  $\boldsymbol{\theta}$ .

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The Escherichia microorganism culture medium is preferably an M9 medium containing, for example, glucose and casamino acid (Miller, Journal of Experiments in Molecular Genetics), 431-433, Cold Spring Harbor Laboratory, New York, 1972. Depending on necessity, the medium may be supplemented with drugs such as 3\$\beta\$-indolyl acrylic acid in order to improve efficiency of the promoter. In the case of the Escherichia host, the cultivation is carried out usually at about 15 to 43 °C for about 3 to 24 hours. As required, aeration and stirring may be applied. In the case of the Bacillus host, the cultivation is carried out usually at about 30 to 40 °C for about 6 to 24 hours. As required, aeration and stirring may be also applied. In the case of the transformant in which the

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Proc. Natl. Acad. Sci. USA, Vol. 77, 4505 (1980)], an SD medium containing 0.5% casamino acid [Bitter, G.A. et al., 20 Proc. Natl. Acad. Sci. USA, Vol. 81, 5330 (1984)], etc. It is preferable that pH of the culture medium is adjusted to be from about 5 to about 8. The cultivation is carried out usually at about 20 to 35 °C for about 24 to 72 hours. As required, aeration and stirring may be applied.

example, a Burkholder minimum medium (Bostian, K.L. et al.,

host is an yeast, the culture medium used may include, for

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referred to as "TM").

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In the case of the transformant in which the host is an insect Association, Vol. 199, 519 (1967)], 199 medium [Proceedings of the culture medium used may include those obtained by suitably T.C.C., Nature, 195, 788 (1962)). It is preferable that pH of In the case of the transformant in which the host is an animal bovine serum and the like to the Grace's insect medium (Grace, cell, the culture medium used may include MEM medium (Science, cultivation is usually carried out at about 27 °C for about 3 to 5 days. As desired, aeration and stirring may be applied. the culture medium is adjusted to be about 6.2 to 6.4. The (1959)], RPMI 1640 medium [Journal of the American Medical Vol. 122, 501 (1952)], DMEM medium (Virology, Vol. 8, 396 adding additives such as passivated (or immobilized) 10% 25 30 35

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the Society of the Biological Medicine, Vol. 73, 1 (1950)], etc. which are containing, for example, about 5 to 20% of fetal calf serum. It is preferable that the pH is from about 6 to about 8. The cultivation is usually carried out at about 30 to 40 °C for about 15 to 60 hours. As required, aeration and stirring may be applied.

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Separation and purification of the G protein coupled receptor protein from the above-mentioned cultures can be carried out according to methods described herein below.

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from the cultured microorganisms or cells, the microorganisms or cells are collected by known methods after the cultivation, suspended in a suitable buffer solution, disrupted by ultrasonic waves, lysozyme and/or freezing and thawing, etc. and, then, a crude extract of the G protein coupled receptor protein is obtained by centrifugation or filtration. Other conventional extracting or isolating methods can be applied. The buffer solution may contain a protein-denaturing agent such as urea or guanidine hydrochloride or a surfactant such as Triton X-100 (registered trademark, hereinafter often

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In case where G protein coupled receptor proteins are secreted into culture media, supernatant liquids are separated from the microorganisms or cells after the cultivation is finished and the resulting supernatant liquid collected by widely known methods. The culture supernatant liquid and extract containing G protein coupled receptor proteins can be purified by suitable combinations of widely known methods for separation, isolation and purification. The widely known methods of separation, isolation and purification may include methods which utilizes solubility, such as salting out or sedimentation with solvents

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size or weight, such as dialysis, ultrafiltration, gel filtration and SDS-polyacrylamide gel electrophoresis, methods utilizing a difference in the electric charge, such as ion-exchange chromatography, methods utilizing specific

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methods which utilizes chiefly a difference in the molecular

electrophoresis, etc. difference in the isoelectric point such as isoelectric high-performance liquid chromatography, and methods utilizing a a difference in the hydrophobic property, such as inverse-phase affinity such as affinity chromatography, methods utilizing

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other salt thereof by known methods or method analogous the protein salt can be converted into a free form or into any receptor protein thus obtained is in a salt form vice versa, analogous thereto. In case where the G protein coupled converted into a salt thereof by known methods or method thus obtained is in a free form, the free protein can be In case where the G protein coupled receptor protein

20 5 formed can be measured by experimenting the coupling The activity of the G protein coupled receptor protein thus arginyl endopeptidase, protein kinase, glycosidase, etc. The protein-modifying enzyme may include trypsin, chymotrypsin, protein-modifying enzyme before or after the purification. can be partly removed therefrom, by the action of a suitable the transformant can be arbitrarily modified or a polypeptide linked immunoassays) using specific antibodies. (or binding) with a ligand or by enzyme immunoassays (enzyme The G protein coupled receptor protein produced by



- receptor protein of the present invention, methods of determining ligands for the G protein coupled
- obtaining an antibody and an antiserum,
- 30 constructing a system for expressing a recombinant receptor
- compounds, developing system and screening pharmaceutical candidate developing a receptor-binding assay system using the above
- receptors which have a similar or analogous structure, designing drugs based upon the comparison with ligands and

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preparing a probe in the analysis of genes and preparing

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- a PCR primer, and
- gene manipulating therapy.

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5 permits various applications including prevention and/or protein coupled receptor protein of the present invention. system which uses a system for expressing a recombinant G blooded animal such as human being by a receptor-binding assay coupled receptor agonist or antagonist specific to a warmtherapy of a variety of diseases. The agonist or antagonist thus screened or characterized In particular, it is allowable to screen a G protein

fragment thereof), G protein coupled receptor protein-encoding coupled receptor proteins, partial peptide thereof (peptide protein according to the present invention. DNAs and antibodies against the G protein coupled receptor Concretely described below are uses of G protein

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20 25 said DNA, peptide fragments or segments thereof (including coupled receptor protein-encoding DNAs according to the present encoding DNA obtained by the screening method for G protein on the usefulness of the G protein coupled receptor proteincells or cell membrane fractions thereof each containing the coupled receptor protein or a peptide fragment thereof"), including their salts, will be referred to as the "G protein partial peptides thereof) or salts thereof (hereinafter, those invention, the G protein coupled receptor proteins encoded by Their various applications are also disclosed herein below. recombinant type G protein coupled receptor protein, etc. As hereunder, more detailed description will be made

(1) Method for Determining Ligands to the G Protein Coupled Receptor Protein

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investigating or determining a ligand to said G protein peptide segment thereof) is useful as a reagent for receptor protein. The G protein coupled receptor protein (or the

determining a ligand to the G protein coupled receptor protein According to the present invention, methods for

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protein or the peptide segment or fragment thereof with the which comprises contacting the G protein coupled receptor compound to be tested are provided.

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The compound to be tested may include not only known polypeptides, galanin, modified derivatives thereof, analogues and human being), etc. For example, said tissue extract, said stimulating activity, etc. and fractionated by relying on the thereof, family members thereof and the like but also tissue adenosine, adrenaline, a - and eta -chemokines (IL-8, GROa , GROB , GRO7 , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, animals (such as mice, rats, swines, cattle, sheep, monkeys leukotrienes, pancreastatins, prostaglandins, thromboxanes, neuropeptides Y, opioids, purine, vasopressins, oxytocins, MCP-3, I-309, MIP1a , MIP-1 $\beta$  , RANTES, etc.), endothelins, extracts, cell culture supernatants, etc. of warm-blooded CGRP (calcitonin gene related peptides), adrenomedullins, enterogastrins, histamine, neurotensins, TRH, pancreatic somatostatins, dopamine, motilins, amylins, bradykinins, cell culture supernatant, etc. is added to the G protein ligands such as angiotensins, bombesins, canavinoids, measurements whereupon a single ligand can be finally coupled receptor protein for measurement of the cell cholecystokinins, glutamine, serotonin, melatonins, VIP (vasoactive intestinal and related peptides),

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stimulating a target cell which comprises binding said compound In one specific embodiment of the present invention, Examples of said cell stimulating activities include promoting peptide segment thereof or in a receptor binding assay system said method for determining the ligand includes a method for receptor protein is constructed and used; and measuring the with the G protein coupled receptor protein either in the presence of the G protein coupled receptor protein or the activity or inhibiting activity on biological responses, in which the expression system for the recombinant type determining a compound or a salt thereof capable of receptor-mediated cell stimulating activity, etc.

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endocellular CAMP, production of endocellular cGMP, production of inositol phosphate, changes in the cell membrane potential, etc. Examples of said compound or salt capable of stimulating acetylcholine, liberation of endocellular  $Ca^{2+}$ , production of phosphorylation of endocellular protein, activation of c-fos, lowering in pH, activation of G protein, cell promulgation, protein include peptides, proteins, nonpeptidic compounds, the cell via binding with the G protein coupled receptor e.g. liberation of arachidonic acid, liberation of synthetic compounds, fermented products, etc.

protein coupled receptor protein or the peptide segment thereof cell stimulating activity, etc. of the test compound to the G receptor protein or the peptide segment thereof is contacted with the test compound, for example, the binding amount, the characteristic feature is that when the G protein coupled In said method for determining the ligand, the

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invention, said methods for determining the ligand includes: In more specific embodiments of the present

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- labeled test compound binding with said protein or salt theregé a method of determining a ligand to a G protein coupled receptor protein, which comprises contacting a labeled test peptide segment thereof, and measuring the amount of the compound with a G protein coupled receptor protein or a or with said peptide fragment or salt thereof;
- the amount of the labeled test compound binding with said cells a method of determining a ligand to a G protein coupled compound with cells containing the G protein coupled receptor protein or the membrane fraction of said cell, and measuring receptor protein, which comprises contacting a labeled test or said cell fraction;

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compound with the G protein coupled receptor protein expressed measuring the amount of the labeled test compound binding with a method of determining a ligand to a G protein coupled receptor protein, which comprises contacting a labeled test on cell membranes by culturing transformants containing the DNA coding for the G protein coupled receptor protein, and

said G protein coupled receptor protein;

protein, and measuring the cell stimulating activity with cells containing the G protein coupled receptor (e.g. promoting or inhibiting activity on biological responses receptor protein, which comprises contacting a test compound a method of determining a ligand to a G protein coupled

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of inositol phosphate, changes in the cell membrane potential, endocellular cAMP, production of endocellular cGMP, production etc.) via the G protein coupled receptor protein; and phosphorylation of endocellular protein, activation of c-fos, acetylcholine, liberation of endocellular  $\mathbf{Ca}^{2+}$ , production of such as liberation of arachidonic acid, liberation of lowering in pH, activation of G protein, cell promulgation, a method of determining a ligand to the G protein coupled

15 20 receptor protein, which comprises contacting a test compound or inhibiting physiological responses such as liberation of cell membrane by culturing transformants containing the DNA with the G protein coupled receptor protein expressed on the measuring the cell stimulating activity (activity for promoting coding for the G protein coupled receptor protein, and

phosphorylation of endocellular protein, activation of c-fos, phosphate, changes in the cell membrane potential, production of endocellular cGMP, production of inositol endocellular Ca2+, production of endocellular cAMP, arachidonic acid, liberation of acetylcholine, liberation of etc.) via the G protein coupled receptor protein. lowering in pH, activation of G protein, cell promulgation,

invention which are provided only for illustrative purposes. determining method of ligands according to the present First, the G protein coupled receptor protein used Described below are specific explanations on the

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protein or a peptide fragment or segment thereof material so far as it contains a G protein coupled receptor for the method for determining the ligand may include any

although it is preferable to express a large amount of G protein coupled receptor proteins in animal cells. (including a partial peptide thereof) or a salt thereof

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v mammalian cells or in insect cells. With respect to the DNA carried out by expressing said protein encoding DNA in protein, the above-mentioned method can be used and it may be fragments or synthetic DNA may be used as well. used although it is not limited thereto. For example, gene fragment coding for the aimed region, complementary DNA may be In the manufacture of the G protein coupled receptor

10 20 express it efficiently, it is preferred that said DNA fragment protein-encoding DNA fragment into host animal cells and to is incorporated into the downstream site of polyhedron belonging to baculovirus, promoters derived from SV40 promoters derived from nuclear polyhedrosis virus promoters, etc. Examinations of the quantity and the quality human heat shock promoters, cytomegalovirus promoters,  $SR\alpha$ promoters derived from retrovirus, metallothionein promoters, of Biochemical Society, vol.267, pages 19555-19559 (1992). described in publications such as Nambi, P. et al: The Journal thereto. For example, they may be conducted by methods se known to those of skill in the art or methods similar of the expressed receptor can be carried out by methods per In order to introduce the G protein coupled receptor

30 products containing G protein coupled receptor proteins which receptor protein or peptide segment thereof may include the ligand, the material containing a G protein coupled art or methods similar thereto, peptide fragments of said G are purified by methods per se known to those of skill in the cell containing said protein, etc. protein coupled receptor protein, membrane fractions of the protein coupled receptor protein, cells containing said G Accordingly, with respect to the determination of

may be carried out by methods per se known to those of skill including glutaraldehyde, formalin, etc. The immobilization containing cell is used in the determining method of the ligand, said cell may be immobilized with binding agents in the art or methods similar thereto. When the G protein coupled receptor protein-

The G protein coupled receptor protein-

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containing cells are host cells expressing the G protein coupled receptor protein. Examples of said host cells are microorganisms such as Escherichia coli, Bacillus subtilis, yeasts, insect cells, animal cells, etc.

of skill in the art or methods similar thereto after disruption via blowing out cells from small nozzles together with applying by Kinematica), a disruption by ultrasonic waves, a disruption components such as phospholipids and membrane proteins derived fractionation of the cell membrane, a fractionation method by centrifuged at a low speed (500 rpm to 3,000 rpm) for a short The cell membrane fraction is a cell membrane-rich a disruption by a Waring blender or a Polytron (manufactured and the resulting precipitate is used as a membrane fraction. fraction which is prepared by methods per se known to those of cells. Examples of cell disruption may include a method (1,500 rpm to 3,000 rpm) usually for 30 minutes to two hours means of centrifugal force such as a fractional centrifugal separation and a density gradient centrifugal separation is a pressure using a French press or the like, etc. In the supernatant liquid is further centrifuged at a high speed Said membrane fraction contains a lot of the expressed G for squeezing cells using a Potter-Elvejem homogenizer, mainly used. For example, disrupted cellular liquid is protein coupled receptor protein and a lot of membrane period (usually, from about one to ten minutes), the from the cells.

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The amount of the G protein coupled receptor protein in the membrane fraction cell containing said G protein coupled receptor protein is preferably  $10^3$   $^{-10}$  molecules per cell or, suitably,  $10^5$  to  $10^7$  molecules per cell. Incidentally, the more the expressed amount, the higher the ligand binding activity (specific activity) per membrane fraction whereby the construction of a highly sensitive screening system becomes possible and, moreover, it may enable us to measure the large amount of samples within the same lot.

In conducting the above-mentioned methods  $\ensuremath{\mathbb{O}}$  to  $\ensuremath{\mathbb{O}}$  wherein ligands capable of binding with the G protein coupled

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receptor protein are determined, a suitable G protein coupled receptor fraction and a labeled test compound are necessary. The G protein coupled receptor fraction is preferably a naturally occurring (natural type) G protein coupled receptor, a recombinant type G protein coupled receptor having the activity equivalent to that of the natural type. Here, the term "activity equivalent to" means the equivalent ligand binding activity, etc.

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Specifically, the determination of ligands capable of binding with G protein coupled receptor proteins is carried out as follows:

First, cells or cell membrane fractions containing the G protein coupled receptor protein are suspended in a buffer suitable for the determining method to prepare the receptor sample in conducting the method of determining the ligand binding with the G protein coupled receptor protein. The buffer may include any buffer such as Tris-HCl buffer or phosphate buffer with pH 4-10 (preferably, pH 6-8), etc., as long as it does not inhibit the binding of the ligand with the receptor. In addition, surface-active agents such as CHAPS, Tween 80 TM (Kao-Atlas, Japan), digitonin, deoxycholate, etc. and various proteins such as bovine serum albumin (BSA), gelatin, milk derivatives, etc. may be added to the buffer with an object of decreasing the non-specific binding.

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Further, a protease inhibitor such as PMSF, leupeptin, E-64 (manufactured by Peptide Laboratory), pepstatin, etc. may be added with an object of inhibiting the decomposition of the receptor and the ligand by protease. A test compound labeled with a predetermined (or certain) amount (5,000 cpm to 500,000 cpm) of [3H], [125], [14C], [35], etc. is made copresent in 0.01 ml to 10 ml of said receptor solution.

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15 20 a reaction tube to which a great excessive amount of the fiber filter or the like, washed with a suitable amount of hours). After the reaction, it is filtered through a glass for 20 minutes to 24 hours (preferably 30 minutes to three The reaction is carried out at 0-50°C (preferably at 4-37°C) unlabeled test compound is added is prepared as well. more than 0 cpm can be selected as a ligand to the G protein binding amount (NSB) from the total binding amount (B) is count (B - NSB) obtained by subtracting the non-specific counter or a gamma-counter. The test compound in which the fiber filter is measured by means of a liquid scintillation the same buffer and the radioactivity remaining in the glass In order to know the non-specific binding amount (NSB), coupled receptor protein of the present invention.

underein ligands capable of binding with the G protein coupled receptor protein are determined, the cell stimulating activity (e.g. the liberation of arachidonic acid, the liberation of acetylcholine, endocellular Ca 2+ liberation, endocellular camp production, the production of insitol phosphate, changes in the cell membrane potential, the phosphorylation of endocellular protein, the activation of c-fos, lowering of pH, the activation of G protein, cell promulgation, etc.) mediated by the G protein coupled receptor protein may be measured by known methods or by the use of commercially available measuring kits. To be more specific, G protein coupled receptor protein-containing cells are at first cultured in a multi-well plate or the like.

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In conducting the determination of ligand, it is substituted with a fresh medium or a suitable buffer which

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experiment, and incubated for certain period after adding a test compound, etc. thereto. Then, the cells are extracted or the supernatant liquid is recovered and the resulting product is determined by each of the methods. When it is difficult to identify the production of the substance (e.g. arachdonic acid) which is to be an index for the cell stimulating activity due to the decomposing enzyme contained in the cell, an assay may be carried out by adding an inhibitor against said decomposing enzyme. With respect to the activity such as an inhibitory action against cAMP production, it may be detected as an inhibitory action against the production of the cells whose fundamental production is increased by forskolin or the like.

The kit used for the method of determining the ligand binding with the G protein coupled receptor protein includes a G protein coupled receptor protein or a peptide fragment thereof, cells containing the G protein coupled receptor protein, a membrane fraction from the cells containing the G protein coupled receptor protein.

Examples of the kit for determining the ligand are

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1. Reagent for Determining the Ligand.

lacktriangle Buffer for Measurement and Buffer for Washing.

The buffering product wherein 0.05% of bovine serum albumin (manufactured by Sigma) is added to Hanks' Balanced Salt Solution (manufactured by Gibco).

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This product may be sterilized by filtration through a membrane filter with a 0.45  $\mu$  m pore size, and stored at 4°C or may be formulated upon use.

30 @ G Protein Coupled Receptor Protein Sample.

CHO cells in which G protein coupled receptor proteins are expressed are subcultured at the rate of 5 x  $10^5$  cells/well in a 12-well plate and cultured at  $37^{\circ}$ C in a humidified 5% CO $_2$ /95% air atmosphere for two days to prepare the earnle

the sample.

Labeled Test Compound.

The compound which is labeled with commercially

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available [  $^3$  H], [  $^{12}$ S], [  $^{14}$ C], [  $^{3}$ S], etc. or labeled with a suitable method.

The product in a state of an aqueous solution is stored at 4°C or at  $-20^{\circ}$ C and, upon use, diluted to 1  $\mu$  M with a buffer for the measurement. In the case of the test compound which is hardly soluble in water, it is dissolved in dimethylformamide, DMSO, methanol, etc.

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Unlabeled Test Compound.

The same compound for the labeled one is prepared in a concentration of 100 to 1,000-fold concentrated state.

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2. Method of Measurement,

 $\oplus$  G protein coupled receptor protein-expressing CHO cells cultured in a 12-well tissue culture plate are washed twice with 1 ml of buffer for the measurement and then 490  $\mu$  1 of buffer for the measurement is added to each well.

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2 Five u l of the labeled test compound is added and the mixture is made to react at room temperature for one hour. For measuring the nonspecific binding amount, 5 u l of the unlabeled test compound is added.

The reaction solution is removed from each well, which is washed with 1 ml of a buffer for the measurement three times. The labeled test compound which is binding with the cells is dissolved in 0.2N NaOH-1% SDS and mixed with 4 ml of a liquid scintillator A (manufactured by Wako Pure Chemical, Japan).

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The ligand which can bind with the G protein coupled receptor protein include substances occurring or existing, for example, in brain, pituitary gland, pancreas, etc. Examples of the ligand are angiotensin, bombesin, canavinoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, VIP (vasoactive

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motilin, amylin, bradykinin, CGRP (calcitonin gene related

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intestinal and related peptide), somatostatin, dopamine,

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Peptide), adrenomedullin, leukotriene, pancreastatin, prostaglandin, thromboxane, thromboxatin, adenosine, adrenaline, a – and  $\beta$  –chemokine (IL-8, GROa, GRO $\beta$ , GRO $\gamma$ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1a, MIP-1 $\beta$ , RANTES, etc.), endothelin, enterogastrin, histamine, neurotensin, TRH, pancreatic polypeptide, galanin, modified derivatives thereof, analogues thereof, etc.

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families. All data including electrophysiological measurements related to purine compounds, and in developing pharmaceuticals. to be a novel human type purinoceptor. It is presumed that it is advantageously useful in efficiently screening for agonists MIN6-derived receptor protein of the present invention (e.g., Protein of the present invention (e.g., SEQ ID NO: 38 and SEQ SEQ ID NO: 56, or proteins encoded by phAH2-17) is considered or antagonists to receptor proteins which control or regulate highly homologous to prinoceptors, it is considered that the Since the receptor protein encoded by pMAH2-17 is  $_l$ PMAH2-17) is a novel purinoceptor subtype. In other words, compound such as ATP. Further, the receptor protein (e.g., the mouse pancreatic eta -cell strain, MIN6-derived receptor functions in the central nervous system or immune system, are supporting that the mouse pancreatic eta -cell strain, it is suggested that the ligand capable of binding with ID NO: 39, or proteins encoded by PMAH2-17) is a purine SEQ ID NO: 38 and SEQ ID NO: 39, or proteins encoded by are strong possibility of a subtype within prinoceptor

(2) Preventive and Therapeutic Agent for of G Protein Conjugated Receptor Protein Deficiency Diseases

If a ligand to the G protein coupled receptor protein is disclosed via the aforementioned method (1), the G protein coupled receptor protein-encoding DNA can be used a preventive and/or therapeutic agent for treating said G protein coupled receptor protein deficiency diseases depending upon the action that said ligand exerts.

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For example, when there is a patient for whom the physiological action of the ligand cannot be expected because

of a decrease in the G protein coupled receptor protein in vivo. of the ligand can be fully achieved by: the amount of the G protein coupled receptor protein in the brain cells of said patient can be increased whereby the action

- v encoding DNA to the patient to express it; or (a) administering the G protein coupled receptor protein-
- deficiency diseases. In an embodiment, it is suggested that therapeutic agent for the G protein coupled receptor protein DNA can be used as a safe and less toxic preventive and Accordingly, the G protein coupled receptor protein-encoding transplanting said brain cells or the like to said patient. DNA into brain cells or the like to express it, followed by the ligands capable of binding with the mouse pancreatic inserting the G protein coupled receptor protein-encoding
- 15 20 autoimmune disease, rheumatoid arthritis, rejection on internal or syndromes in connection with purine ligand compounds. ATP. Therefore, the disease to be treated may include diseases or proteins encoded by phAH2-17) are purine compounds such as organ transplant, hypertension, diabetes, cystic fibrosis, Examples of such diseases may include cancer, immunodeficiency, receptor protein of the present invention (e.g., SEQ ID NO: 56 encoded by pMAH2-17) and further with the human-derived invention (e.g., SEQ ID NO: 38 and SEQ ID NO: 39, or proteins eta -cell strain, MIN6-derived receptor protein of the present
- (3) Preventive and Therapeutic Pharmaceutical Composition for Deficiency Diseases Human-Derived G Protein Conjugated Receptor Protein

hypotension, incontinence of urine, pain, etc.

diseases of said human-derived G protein coupled receptor protein-encoding DNA is screened and a ligand for said humanprotein depending upon the action that said ligand exhibits. an agent for the prevention or therapy of the deficiency protein coupled receptor protein-encoding DNA can be used as using the above-mentioned method (1), the human-derived G derived G protein coupled receptor protein can be clarified If the human-derived G protein coupled receptor

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of a decrease in the G protein coupled receptor protein in vivo. the amount of the G protein coupled receptor protein in the physiological action of the ligand cannot be expected because For example, when there is a patient for whom the

- of the ligand can be fully achieved by: brain cells of said patient can be increased whereby the action
- encoding DNA to the patient to express it; or (a) administering the G protein coupled receptor protein-
- 5 15 DNA into brain cells or the like to express it, followed by transplanting said brain cells or the like to said patient. therapeutic agent for the G protein coupled receptor protein DNA can be used as a safe and less toxic preventive and Accordingly, the G protein coupled receptor protein-encoding (b) inserting the G protein coupled receptor protein-encoding deficiency diseases.

20 virus vector, etc. followed by subjecting the product vector DNA is used as the above-mentioned agent, said DNA may be used include tablets (sugar-coated if necessary), capsules, pharmaceutical compositions or formulations. Oral formulations parenterally, by inhalation spray, rectally, or topically as to a conventional means. Thus, it may be administered orally retrovirus vector, adenovirus vector, adenovirus-associated alone or after inserting it into a suitable vector such as When the G protein coupled receptor protein-encoding

- 25 which is required for preparing generally approved the DNA of the present invention is admixed in a unit dose form or in other pharmaceutically acceptable liquid. For example, injections such as an aseptic solution or a suspension in water elixirs, microcapsules, etc. Parenteral formulations include
- ü ű etc. whereupon the preparation can be manufactured. The amount diluents, fillers, vehicles, antiseptics, stabilizers, binders, pharmaceutical preparations together with a physiologically such an extent that the suitable dose within an indicated of the effective component in those preparations is to be in range is achieved. acceptable carriers, flavoring agents, adjuvants, excipients,
- Examples of the additives which can be admixed in the

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tablets, capsules, etc. are binders such as gelatin, corn starch, tragacanth and gum arabicum; fillers such as crystalline cellulose; swelling agents such as corn starch, gelatin and alginic acid; lubricating agents such as magnesium stearate; sweetening agents such as sucrose, lactose and saccharine; and flavoring agents such as pepper mint, akamono oil and cherry. When the unit dose form of the preparation is a capsule, a liquid carrier such as fat/oil may be further added in addition of the above-mentioned types of materials. The aseptic composition for injection may be formulated by conventional practices for the preparations such as that the active substance in a vehicle such as water for injection is dissolved or suspended in naturally occurring plant oil such as sesame oil and palm oil.

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sodium chloride, etc.) wherein a suitable auxiliary solubilizers glucose and other auxiliary agents (e.g. D-sorbitol, D-mannitol, physiological saline solution and isotonic solutions containing active agent (e.g. Polysorbate  $80^{\,\mathrm{TM}}$  , HCO-50, etc.), etc. may be therefore, it can be administered to warm-blooded animals (e.g., soybean oil, etc. wherein benzyl benzoate, benzyl alcohol, etc. phenol, etc.), antioxidants, etc. may be admixed therewith too. The prepared injection solution is filled in suitable ampoules. propylene glycol polyethylene glycol, etc.), nonionic surfacebuffers (e.g. phosphate buffer, sodium acetate buffer, etc.), jointly used. Examples of an oily liquid include sesame oil, may be jointly used as auxiliary solubilizers. In addition, polyethylene glycol, etc.), stabilizers (e.g. benzyl alcohol The preparation prepared as such is safe and less toxic and, Examples of an aqueous liquid for injection are a hydrochloride, etc.), stabilizers (e.g. human serum albumin, rat, rabbit, sheep, swine, cattle, cat, dog, monkey, human such as alcohol (e.g. ethanol, etc.), polyalcohol (e.g. analgesic agents (e.g. benzalkonium chloride, procaine beings, etc.).

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Specific dose levels of said DNA may vary depending upon a variety of factors including the activity of drugs employed, the age, body weight, general health, sex, diet,

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time of administration, route of administration, drug combination, and the severity of the symptom. In the case of oral administration, it is usually about 0.1-100 mg, preferably about 1.0-50 mg or, more preferably, about 1.0-20 mg per day for adults (as 60 kg). When it is administered parenterally, its dose at a time may vary depending upon the object (patient) to be administered, organs to be administered, symptoms, administering methods, etc. but, in the case of injections, is usually convenient to give by an intravenous route in an amount of about 0.01-30 mg, preferably about 0.1-20 mg or, more preferably, about 0.1-10mg per day to adults (as 60 kg). In the case of other animals, the dose calculated for 60 kg may be administered as well.

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(4) Quantitative Determination of Ligand to the G Protein Conjugated Receptor Protein of the Present Invention.

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The G protein coupled receptor protein or a peptide fragment thereof has a binding property to ligand and, therefore, it is capable of determining quantitatively an amount of ligands in vivo with good sensitivity.

This quantitative determination may be carried out by, for example, combining with a competitive method. Thus, samples to be determined is contacted with G protein coupled receptor proteins or peptide fragments thereof so that the ligand concentration in said sample can be determined. In one embodiment of the quantitative determination, the

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protocols described in the following ① and ② or the methods similar thereto may be used:
① Hiroshi Irie (ed): "Radioimmunoassay" (Kodansha, Japan,

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1974); and

Hiroshi Irie (ed): "Radioimmunoassay, Second Series"
(Kodansha, Japan, 1979).

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- (5) Screening of Compound Inhibiting the Binding of Ligand with the G Protein Conjugated Receptor Protein of the Present Invention.
- G Protein coupled receptor proteins or peptide

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peptide fragments thereof are constructed and receptor binding assay systems using said expression system are used. In these for recombinant type G Protein coupled receptor proteins or fragments thereof are used. Alternatively, expression systems

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acid, liberation of acetylcholine, endocellular  $\operatorname{\mathsf{Ca}}^{2+}$  liberation, assay systems, it is possible to screen compounds (e.g. physiological reactions including liberation of arachdonic (e.g. activity of promoting or activity of inhibiting protein. Such a compound includes a compound exhibiting a binding of a ligand with the G protein coupled receptor tissue extracts, etc.) or salts thereof which inhibits the fermented products, cell extracts, plant extracts, animal peptides, proteins, nonpeptidic compounds, synthetic compounds, protein coupled receptor-mediated cell stimulating activity

15 20 receptor-agonist"), a compound free of such a cell stimulating cell promulgation, etc.) (so-called "G protein coupled activation of c-fos, lowering of pH, activation of G protein, potential, phosphorylation of endocellular proteins, production of inositol phosphate, changes in cell membrane endocellular cAMP production, endocellular cGMP production, activity (so-called "G protein coupled receptor-antagonist"),

characterized in comparing the following two cases: with a G protein coupled receptor protein or a salt thereof, protein coupled receptor protein or salt thereof, or a peptide (i) the case wherein the ligand is contacted with the G screening a compound which inhibits the binding of a ligand fragment thereof or a salt thereof; and Thus, the present invention provides a method of

(ii) the case wherein the ligand is contacted with a mixture of peptide fragment or salt thereof and said test compound. the G protein coupled receptor protein or salt thereof or the In said screening method, one characteristic feature

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of the ligand, etc. are measured in the case where (i) the the peptide fragment thereof, the cell stimulating activity of the present invention resides in that the amount of the ligand bonded with said G protein coupled receptor protein or

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coupled receptor protein encoding DNA, the amounts of the

labeled ligand binding with said G protein coupled receptor

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and then compared therebetween. and the test compound are contacted with the G protein coupled peptide fragments thereof and in the case where (ii) the ligand receptor protein or the peptide fragment thereof, respectively ligand is contacted with G protein coupled receptor proteins or

invention, the following is provided: In one more specific embodiment of the present

5 protein or a peptide fragment thereof, the amounts of the compound are contacted with a G protein coupled receptor peptide fragment thereof and when a labeled ligand and a test is contacted with a G protein coupled receptor protein or a receptor protein, characterized in that, when a labeled ligand inhibits the binding of a ligand with a G protein coupled a method of screening a compound or a salt thereof which

15 labeled ligand bonded with said protein or peptide fragment thereof or salt thereof are measured and compared; a method of screening a compound or a salt thereof which

20 is contacted with cells containing G protein coupled receptor receptor protein, characterized in that, when a labeled ligand fraction of said cells, the amounts of the labeled ligand proteins or a membrane fraction of said cells and when a inhibits the binding of a ligand with a G protein coupled containing G protein coupled receptor proteins or a membrane labeled ligand and a test compound are contacted with cells

25 binding with said protein or peptide fragment thereof or salt thereof are measured and compared;

G protein coupled receptor protein encoding DNA and when a on the cell membrane by culturing a transformant containing a is contacted with G protein coupled receptor proteins expressed receptor protein, characterized in that, when a labeled ligand inhibits the binding of a ligand with a G protein coupled membrane by culturing a transformant containing a G protein protein coupled receptor proteins expressed on the cell labeled ligand and a test compound are contacted with G a method of screening a compound or a salt thereof which

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protein are measured and compared;

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physiological responses including liberation of arachdonic acid, when the G protein coupled receptor protein-activating compound a method of screening a compound or a salt thereof which containing the G protein coupled receptor protein-encoding DNA, a method of screening a compound or a salt thereof which coupled receptor protein-activating compound (e.g. a ligand to the resulting G protein coupled receptor protein-mediated cell coupled receptor protein-mediated cell stimulating activities protein coupled receptor proteins expressed on cell membranes stimulating activities (activities of promoting or activities activation of c-fos, lowering of pH, activation of G protein, to the G protein coupled receptor protein) is contacted with expressed on the cell membrane by culturing the transformant receptor protein-activating compound and a test compound are the G protein coupled receptor protein) is contacted with G coupled receptor protein-activating compound (e.g. a ligand endocellular cAMP production, endocellular cGMP production, protein coupled receptor proteins, the resulting G protein production of inositol phosphate, changes in cell membrane inhibits the binding of a ligand with a G protein coupled and a test compound are contacted with cells containing G (e.g. activities of promoting or activities of inhibiting liberation of acetylcholine, endocellular  $\mathbb{C}^{2^+}$  liberation, receptor protein-encoding DNA and when a G protein coupled receptor protein, characterized in that, when a G protein receptor protein, characterized in that, when a G protein inhibits the binding of a ligand with a G protein coupled cells containing G protein coupled receptor proteins and cell promulgation, etc.) are measured and compared; and culturing transformants containing G protein coupled contacted with the G protein coupled receptor protein potential, phosphorylation of endocellular proteins,

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membrane potential, phosphorylation of endocellular proteins, activation of c-fos, lowering of pH, activation of G protein, and cell promulgation) are measured and compared.

agonist or antagonist had to be screened by, first, obtaining a Besides, it is allowable to evaluate whether the compound that making sure whether the candidate compound really inhibits the it is allowable to efficiently screen a compound that inhibits Before the G protein coupled receptor protein of the present invention was obtained, the G protein coupled receptor the binding between a ligand and a G protein coupled receptor. binding between human G protein coupled receptor proteins and protein-containing cells, tissues or cell membrane fractions there is no need of effecting the primary screening, whereby derived from rat or the like (primary screening) and, then, antagonists to the desired receptor proteins. By using the is screened is a G protein coupled receptor agonist or a G human-derived G protein coupled receptor protein, however, inevitably exist when the cells, the tissues or the cell ligands (secondary screening). Other receptor proteins candidate compound by using G protein coupled receptor membrane fractions are used as they are, whereby they intrinsically make it difficult to screen agonists or protein coupled receptor antagonist.

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Specific explanations of the screening method will be given as hereunder.

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First, with respect to the G protein coupled receptor protein used for the screening method of the present invention, any product may be used so far as it contains G protein coupled receptor proteins or peptide fragment thereof although the use of a membrane fraction of mammalian organs is suitable. However, human organs is extremely hardly available and, accordingly, G protein coupled receptor proteins which are expressed in a large amount using a recombinant are suitable for the screening.

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In the manufacture of the G protein coupled receptor protein, the above-mentioned method can be used and it may be carried out by expressing the DNA coding for said protein in

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arachdonic acid, liberation of acetylcholine, endocellular  $\mathsf{Ca}^{2^+}$ 

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of inhibiting physiological responses such as liberation of

production, production of inositol phosphate, changes in cell

liberation, endocellular cAMP production, endocellular cGMP

gene fragments or synthetic DNA may be used as well. used although it is not limited thereto. Thus, for example, fragment coding for the target region, complementary DNA may be mammalian cells or in insect cells. With respect to the DNA

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derived from SV40, promoter of retrovirus, metallothionein nuclear polyhedrosis virus belonging to baculovirus, promoter express it efficiently, it is preferred that said DNA fragment protein-encoding DNA fragment into host animal cells and to described in publications such as Nambi, P. et al.: The thereto. For example, they may be conducted by the method methods per se or modified methods substantially analogous quality of expressed receptors can be carried out by known SRa promoter, etc. Examinations of the quantity and the promoter, human heat shock promoter, cytomegalovirus promoter, is incorporated into the downstream of polyhedron promoter of Journal of Biochemical Society, vol.267, pages 1955-19559 In order to introduce the G protein coupled receptor

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containing a G protein coupled receptor protein or a peptide membrane fraction of the cell containing said protein, etc. methods per se, or a cell containing said protein or a cell coupled receptor protein fragment which is purified by known which is purified by known methods per se or a G protein fragment thereof may be a G protein coupled receptor protein Accordingly, in the screening method, the substance

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or modified methods substantially analogous thereto. containing cells are used in the screening method, said cells may be immobilized with glutaraldehyde, formalin, etc. The immobilization may be carried out by known methods per se When the G protein coupled receptor protein-

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animal cells such as CHO cell and COS cell, etc. cells are host cells expressing the G protein coupled receptor protein. Examples of said host cells may include Escherichia coli, Bacillus subtilis, yeasts, insect cells, The G protein coupled receptor protein-containing

lot of cell membranes prepared by known methods per se or Cell membrane fractions are fractions which contain a 35

10 v or crushing by means of ultrasonic wave, disrupting by blowing blender or a Polytron (manufactured by Kinematica), disrupting Potter-Elvejem homogenizer, disrupting or crushing by a Waring the cell may include methods by squeezing the cells with a disrupting or crushing the cells. Examples of disruptions of modified methods substantially analogous thereto after cells is centrifuged at a low speed (500 rpm to 3,000 rpm) for a fractional centrifugal separation and a density gradient fractionation techniques by means of centrifugal force such as Fractionation of the cell membrane is carried out mainly by pressure with a French press or the like, etc. out the cells from small nozzles together with applying a centrifugal separation. For example, disrupted liquid of

15 20 supernatant liquid is further centrifuged at a high speed a short period (usually, from about one to ten minutes), the and the resulting precipitate is used as a membrane fraction. phospholipids and membrane proteins derived from the cells. coupled receptor proteins and membrane components such as Said membrane fraction contains a lot of expressed G protein (1,500 rpm to 3,000 rpm) usually for 30 minutes to two hours

25 8  $10^5$  to  $10^\prime$  molecules per cell. Incidentally, the more the preferably 103 -108 molecules per cell or, suitably, in the cell membrane fraction obtained from the cell is in the G protein coupled receptor protein-containing cell and amount of samples in the same lot. possible and, moreover, it is possible to measure the large construction of a highly sensitive screening system is expressed amount, the higher the ligand binding activity (specific activity) per membrane fraction whereby the The amount of the G protein coupled receptor protein

G protein coupled receptors (natural type G protein coupled receptor fraction, it is preferred to use naturally occurring a suitable G protein coupled receptor fraction and a labeled of the ligand with the G protein coupled receptor protein, for screening the compound capable of inhibiting the binding ligand are necessary. With respect to the G protein coupled In conducting the above-mentioned methods  ${\mathbb Q}$  to  ${\mathbb G}$ 

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type G protein coupled. Here the term "activity equivalent to" fractions with the activity equivalent to that of the natural means the same ligand binding activity, or the substantially receptors) or recombinant type G protein coupled receptor eguivalent ligand binding activity.

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With respect to the labeled ligand, it is possible to use labeled ligands, labeled ligand analogized compounds, etc. For example, ligands labeled with  $[^3\mathrm{H}]$ ,  $[^{125}\mathrm{I}]$ ,  $[^{14}\mathrm{C}]$ ,  $[^{35}\mathrm{S}]$ , etc. and other labeled substances may be utilized.

With respect to the buffer, any buffer such as Tris-HCl buffer or phosphate buffer of pH 4-10 (preferably, pH 6-8) which does not inhibit the binding of the ligand with the receptor may be suspended in a buffer which is suitable for the determining Specifically, G protein coupled receptor proteinscreening for a compound which inhibits the binding of the method to prepare the receptor sample in conducting the containing cells or cell membrane fractions are first ligand with the G protein coupled receptor protein.

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said receptor solution and, at the same time, 10  $^{-4}$  M to 10  $^{-10}$  M the nonspecific binding amount (NSB), a reaction tube to which Peptide Laboratory, Japan), pepstatin, etc. may be added with an object of inhibiting the decomposition of the receptor and the ligand by protease. A labeled ligand in a certain amount of a test compound is made copresent. In order to determine a great excessive amount of unlabeled test compounds is added In addition, a surface-active agent such as CHAPS, Tween 80 (Kao-Atlas, Japan), digitonin, deoxycholate, etc. and/or various proteins such as bovine serum albumin (BSA), gelatine, etc. may be added to the buffer with an object of (5,000 cpm to 500,000 cpm) is added to 0.01 ml to 10 ml of decreasing the nonspecific binding. Further, a protease inhibitor such as PMSF, leupeptin, E-64 (manufactured by is prepared as well.

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The reaction is carried out at 0-50°C (preferably at 4-37°C) for 20 minutes to 24 hours (preferably 30 minutes to three hours). After the reaction, it is filtered through a

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Supposing that the count ( $\mathbf{B}_0$  - NSB) obtained by subtracting the retained in the glass fiber filter, etc. is measured by means glass fiber filter, a filter paper, or the like, washed with a suitable amount of the same buffer and the radioactivity of a liquid scintillation counter or a gamma-counter.

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amount (NSB) from the total binding amount (B) is, for example,  $(\mathtt{B}_{\mathsf{A}})$  wherein an antagonizing substance is not present is set at nonspecific binding amount (NSB) from the total binding amount less than 50% may be selected as a candidate ligand to the G 1008, the test compound in which the specific binding amount (B - NSB) obtained by subtracting the nonspecific binding protein coupled receptor protein of the present invention.

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phosphorylation of endocellular proteins, activation of c-fos, lowering of pH, activation of G protein and cell promulgation, for screening the compound which inhibits the binding of the  $\mathsf{Ca}^{2^+}$  liberation, endocellular CAMP production, production of In conducting the above-mentioned methods @ to \$ commercially available measuring kits. To be more specific,( arachidonic acid, liberation of acetylcholine, endocellular protein coupled receptor protein-mediated cell stimulating G protein coupled receptor protein-containing cells are at insitol phosphate, changes in the cell membrane potential, ligand with the G protein coupled receptor protein, the G inhibiting physiological responses such as liberation of etc.) may be measured by known methods or by the use of activity (e.g. activities of promoting or activities of first cultured in a multiwell plate or the like.

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production of the index substance (e.g. arachidonic acid, etc.) adding a test compound, etc. thereto. The resultant cells are by each of the methods. When it is difficult to identify the In conducting the screening, it is substituted with a suitable buffer which does not show toxicity to fresh media which is to be an index for the cell stimulating activity due to the presence of decomposing enzymes contained in the cell, resulting product is determined, preferably quantitatively, or cells in advance, incubated for a certain period after extracted or the supernatant liquid is recovered and the

an assay may be carried out by adding an inhibitor against said decomposing enzyme. With respect to the activities such as an inhibitory action against cAMP production, it may be detected as an inhibitory action against the cAMP production in the cells whose fundamental production has been increased by forskolin or the like.

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In conducting a screening by measuring the cell stimulating activity, cells in which a suitable G protein coupled receptor protein is expressed are necessary. Preferred G protein coupled receptor protein-expressing cells are naturally occurring G protein coupled receptor protein (natural type G protein coupled receptor protein cell lines or strains (e.g. mouse pancreatic \$\beta\$ cell line, MIN6, etc.), the above-mentioned recombinant type G protein coupled receptor protein groupled receptor protein are protein protein.

Examples of the test compound includes peptides, proteins, non-peptidic compounds, synthesized compounds, fermented products, cell extracts, plant extracts, animal tissue extracts, serum, blood, body fluid, etc. Those compounds may be novel or known.

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A kit for screening the compound which inhibits the binding of the ligand with the G protein coupled receptor protein or a salt thereof of the present invention comprises a G protein coupled receptor protein or a peptide fragment thereof, or G protein coupled receptor protein-containing cells or cell membrane fraction thereof.

Examples of the screening kit include as follows:

- Reagent for Determining Ligand.
- Buffer for Measurement and Buffer for Washing.

  The product therein 0 05% of hoving serum all

  The product therein 0 05% of hoving serum all

  The product therein 0 05% of hoving serum all

  The product therein 0 05% of hoving serum all

  The product therein 0 05% of hoving serum all

  The product the

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The product wherein 0.05% of bovine serum albumin (manufactured by Sigma) is added to Hanks' Balanced Salt Solution (manufactured by Gibco).

This may be sterilized by filtration through a membrane filter with a 0.45  $\mu$  m pore size, and stored at 4°C or may be prepared upon use.

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② Sample of G Protein Conjugated Receptor Protein.

CHO cells in which a G protein coupled receptor protein is expressed are subcultured at the rate of 5 x 10 cells/well in a 12-well plate and cultured at 37°C with a 5% CO<sub>2</sub>

and 95% air atomosphere for two days to prepare the sample.

The ligand which is labeled with commercially available [  $^3\text{H}\text{], [}^{125}\text{I], [}^{14}\text{C], [}^{35}\text{S], etc.}$ 

The product in a state of an aqueous solution is stored at 4°C or at -20°C and, upon use, diluted to 1  $\mu$  M with a buffer for the measurement.

Standard Ligand Solution.

Ligand is dissolved in PBS containing 0.1% of bovine serum albumin (manufactured by Sigma) to make 1 mM and stored

Method of the Measurement.

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① CHO cells are cultured in a 12-well tissue culture plate to express G protein coupled receptor proteins. The G protein coupled receptor protein-expressing CHO cells are washed with 1 ml of buffer for the measurement twice. Then 490  $\mu$  1 of

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buffer for the measurement is added to each well.

② Five u l of a test compound solution of  $10^{-3}$  to  $10^{-10}$  M is added, then 5 u l of a labeled ligand is added and is made to react at room temperature for one hour. For knowing the non-specific binding amount, 5 u l of the ligand of

10<sup>-3</sup> M is added instead of the test compound.

① The reaction solution is removed from the well, which is washed with 1 ml of buffer for the measurement three times.

The labeled ligand binding with the cells is dissolved in 0.2N NaOH-1% SDS and mixed with 4 ml of a liquid scintillator A (manufactured by Wako Pure Chemical, Japan).

 Radioactivity is measured using a liquid scintillation counter (manufactured by Beckmann) and PMB (percent of maximum binding) is calculated by the following expression:

 $PMB = [(B - NSB)/(B_0 - NSB)] \times 100$ 

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Value when a sample is added

Percent of maximum binding

PMB:

NSB: Nonspecific binding

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B, : Maximum binding

The compound or a salt thereof obtained by the screening method or by the screening kit is a compound which inhibits the binding of a ligand with a G protein coupled receptor protein and, more particularly, it is a compound having a cell stimulating activity mediated via a G protein coupled receptor or a salt thereof (so-called "G protein coupled receptor agonist") or a compound having no said stimulating activity (so-called "G protein coupled receptor antagonist"). Examples of said compound are peptides, proteins, non-peptidic compounds, synthesized compounds, fermented products, etc. and the compound may be novel or

Said G protein coupled receptor agonist has the same physiological action as the ligand to the G protein coupled receptor protein has and, therefore, it is useful as a safe and less toxic pharmaceutical composition depending upon said ligand activity.

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On the other hand, said G protein coupled receptor antagonist is capable of inhibiting the physiological activity of the ligand to the G protein coupled receptor protein and, there fore, it is useful as a safe and less toxic pharmaceutical composition for inhibiting said ligand activity.

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It is also strongly suggested that agonists and/or antagonists related to the receptor encoded by pMAH2-17 obtained in Example 19 and/or the receptor encoded by phAH2-17 obtained in Example 21 would be useful in therapeutic or prophylactic treatment of diseases or syndromes in connection with purine ligand compounds or related analogues. It is expected that the agonists of the receptor encoded by pMAH2-17 and/or of the receptor encoded by phAH2-17 and/or of the receptor encoded by phAH2-17 and immunomodulator or an antitumor agent, in addition they are

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useful in therapeutically or prophylactically treating hypertension, diabetes, cystic fibrosis, etc. It is still expected that the antagonists of the receptor encoded by pMAH2-17 and/or of the receptor encoded by pMAH2-17 are useful as hypotensive agents, analgesics, agents for therapeutically or prophylactically treating incontinence of urine, etc. With regard to purinoceptors, the mutation of conserved basic amino acid residues in the 6th or 7th putative transmembrane domain of purinoceptors introduces alteration into the receptor's responses to ATP (J. Biol. Chem., Vol. 270(9), pp. 4185-4188 (1995)). It is suggested that ATP is related to blood pressure control and circular systems via receptors (Circulation Research, Vol. 58(3), pp. 319-330 (1986)) and that ATP and purinoceptors are closely related (Am. Phys. Soc., pp. C577-C606 (1993).

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compositions or formulations (e.g. powders, granules, tablets, comprise at least one such compound alone or in admixture with liquid. The pharmaceutical compositions or formulations may excipients and/or diluents. The pharmaceutical compositions For example, said compound or the salt thereof is mixed in a the screening method or by the screening kit is used as the suspensions, solutions, etc.). For example, it may be used When the compound or the salt thereof obtained by suspension in water or in other pharmaceutically acceptable above-mentioned pharmaceutical composition, a conventional capsules, elixiers, microcapsules, etc. or by a parenteral unit dose form which is required for preparing a generally pharmaceutically acceptable carriers, adjuvants, vehicles, cam be formulated in accordance with conventional methods. thereof may be orally, parenterally, by inhalation spray, means may be applied therefor. The compound or the salt pills, capsules, injections, syrups, emulsions, elixirs, by an oral route as tablets (sugar-coated if necessary), rectally, or topically administered as pharmaceutical route as injections such as an aseptic solution or a approved pharmaceutical preparations together with a

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agents (fragrances), fillers, vehicles, antiseptics, within an indicated range is achieved. preparations is to be in such an extent that the suitable dose manufactured. An amount of the effective component in those stabilizers, binders, etc. whereupon the preparation can be physiologically acceptable carriers, flavoring and/or perfuming

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starch, tragacanth and gum arabicum; fillers such as crystalline cellulose; swelling agents such as corn starch, tablets, capsules, etc. are binders such as gelatin, corn antioxidants such as ascorbic acid, a -tocopherol and cysteine; saccharine; preservatives such as parabens and sorbic acid; gelatin and alginic acid; lubricants such as magnesium fragrances such as peppermint, akamono oil and cherry; stearate; sweetening agents such as sucrose, lactose and

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be further added besides the above-mentioned types of preparation is a capsule, a liquid carrier such as fat/oil may polymers, glyceride, lactide,etc. When the unit form of the pectin, collagen, casein, albumin, synthetic or semi-synthetic include mannitol, maltitol, dextran, agar, chitin, chitosan, disintegrants; buffering agents; etc. Other additives may preparations such as that the active substance in  $\cdot a$  vehicle materials. The aseptic composition for injection may be such as water for injection is dissolved or suspended in a formulated by a conventional technique or practice for the

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solubilizers such as alcohol (e.g. ethanol, etc.), polyalcohol D-mannitol, sodium chloride, etc.) wherein a suitable auxiliary containing glucose and other auxiliary agents (e.g. D-sorbitol, physiological saline solution and isotonic solutions sesame oil, soybean oil, etc. may be exemplified wherein etc. may be jointly used. In the case of the oily liquid, surface-active agent (e.g. Polysorbate 80°, (e.g. propylene glycol, polyethylene glycol, etc.), nonionic ', HCO-50, etc.),

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benzyl benzoate, benzyl alcohol, etc. may be jointly used as

auxiliary solubilizers.

naturally occurring plant oil such as sesame oil and palm oil. Examples of the additives which can be admixed in the Examples of an aqueous liquid for the injection are a

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cattle, cats, dogs, monkeys, human being, etc. compounded therewith too. The prepared injection solution is (e.g. benzyl alcohol, phenol, etc.), antioxidants, etc. may be human serum albumin, polyethylene glycol, etc.), stabilizers chloride, procaine hydrochloride, etc.), stabilizers (e.g. acetate buffer, etc.), analgesic agents (e.g. benzalkonium to warm-blooded mammals such as rats, rabbits, sheep, swines, is safe and less toxic and, therefore, it can be administered filled in suitable ampoules. The formulation prepared as such In addition, buffers (e.g. phosphate buffer, sodium

the age, body weight, general health, sex, diet, time of drug combination, and the severity of the particular disease administration, route of administration, rate of excretion, factors including the activity of specific compounds employed, particular patient will be employed depending upon a variety of vary depending upon the symptom. Specific dose levels for any preferably, about 1.0-20 mg per day for adults (as 60 kg). usually about 0.1-100 mg, preferably about 1.0-50 mg or, more undergoing therapy. In the case of oral administration, it is 0.01-30 mg, preferably about 0.1-20 mg or, more preferably, intravenous, intramuscular, intraperitoneal injections, or administered, symptoms, administering methods, etc. The term vary depending upon the object to be administered, organs to be When it is administered parenterally, its dose at a time may other animals, the dose calculated for 60 kg may be about 0.1-10 mg per day to adults (as 60 kg). In the case of convenient to give by an intraveous route in an amount of about infusion techniques. In the case of injections, it is usually "parenteral" as used herein includes subcutaneous injections, Dose levels of said compound or the salt thereof may

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administered as well.

(6) Manufacture of Antibody or Antiserum against the G Protein Its Peptide Fragment or Its Salt. Coupled Receptor Protein of the Present Invention,

antibody) and antisera against the G protein coupled receptor Antibodies (e.g. polyclonal antibody and monoclonal

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protein or salt thereof of the present invention or against the peptide fragment of the G protein coupled receptor protein or salt thereof of the present invention may be manufactured by antibody or antiserum-manufacturing methods per se known to those of skill in the art or methods similar thereto, using the G protein coupled receptor protein or its salt of the present invention or the peptide fragment of the G protein coupled receptor protein or its salt of the present invention.

For example, monoclonal antibodies can be manufactured by the method as given below.

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[Preparation of Monoclonal Antibody]

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(a) Preparation of Monoclonal Antibody-Producing Cells.

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The G protein coupled receptor protein of the present invention or its salt or the peptide fragment of the G protein coupled receptor protein of the present invention or its salt (hereinafter, may be abbreviated as the "G protein coupled receptor protein") is administered to warm-blooded animals either solely or together with carriers or diluents to the site where the production of antibody is possible by the administration. In order to potentiate the antibody productivity upon the administration, complete Freund's adjuvants or incomplete Freund's adjuvants may be administered. The administration is usually carried out once every two to six weeks and two to ten times in total.

Examples of the applicable warm-blooded animals are monkeys, rabbits, dogs, guinea pigs, mice, rats, sheep, goats and chickens and the use of mice and rats is preferred.

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In the preparation of the cells which produce monoclonal antibodies, an animal wherein the antibody titer is noted is selected from warm-blooded animals (e.g. mice) immunized with antigens, then spleen or lymph node is collected after two to five days from the final immunization and antibody-producing cells contained therein are fused with myeloma cells to give monoclonal antibody-producing hybridomas. Measurement of the antibody titer in antisera may, for example, be carried out by reacting a labeled

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G protein coupled receptor protein (which will be mentioned later) with the antiserum followed by measuring the binding activity of the labeling agent with the antibody.

The operation for fusing may be carried out, for example, by a method of Koehler and Milstein (Nature, 256, 495, 1975).

Examples of the fusion accelerator are polyethylene glycol (PEG), Sendai virus, etc. and the use of PEG is preferred.

Examples of the myeloma cells are NS-1, P301, SP2/q AP-1, etc. and the use of P301 is preferred. The preferred fusion ratio of the numbers of antibody-producing cells used (spleen cells) to the numbers of myeloma cells is within a range of about 1:1 to 20:1. When PEG (preferably, PEG 1000 to PEG 6000) is added in a concentration of about 10-80% followed by incubating at 20-40°C (preferably, at 30-37°C) for one to ten minutes, an efficient cell fusion can be

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anti-G protein coupled receptor monoclonal antibodies bound on monoclonal antibodies bonded with the solid phase is detected. protein coupled receptor labeled with a radioactive substance is used when the cells used for the cell fusion are those of the solid phase are detected; or a supernatant liguid of the immunoglobulin antibody (anti-mouse immunoglobulin antibody enzyme or the like, or protein A is added thereto and then antibody. For example, a supernatant liquid of hybridoma mouse) which is labeled with a radioactive substance, an or an enzyme is added and anti-G protein coupled receptor Various methods may be applied for screening a hybridoma which produces anti-G protein coupled receptor anti-immunoglobulin or protein A is adsorbed, then the G which the G protein coupled receptor protein antigen is culture is added to a solid phase (e.g. microplate) to adsorbed either directly or with a carrier, then antihybridoma culture is added to the solid phase to which

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Selection and cloning of the anti-G protein coupled receptor monoclonal antibody-producing hybridoma may be carried out by methods per se known to those of skill in the art or methods similar thereto. Usually, it is carried out in a

medium for hybridoma culturing (SFM-101; Nissui Seiyaku, Japan). fetal calf serum (FCS) , a GIT medium (Wako Pure Chemical, Co., Ltd., Japan) containing 1-20% (preferably 10-20%) of of the medium are an RPMI 1640 medium (Dainippon Pharmaceutical selection, for the cloning and for the growth, any medium may aminopterin and thymidine). With respect to a medium for the measured by the same manner as in the above-mentioned The culturing temperature is usually 20-40°C and, preferably, Japan) containing 1-20% of fetal calf serum and a serum-free be used so far as hybridoma is able to grow therein. Examples medium for animal cells, containing HAT (hypoxanthine measurement of the antibody titer of the anti-G protein coupled titer of the supernatant liquid of the hybridoma culture may be three weeks and, preferably, one to two weeks. The culturing receptor in the antiserum. is usually carried out in 5% carbon dioxide gas. The antibody The culturing time is usually from five days to

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The cloning can be usually carried out by methods known per se such as techniques in semi-solid agar and limiting dilution. The cloned hybridoma is preferably cultured in modern serum-free culture media to obtain optimal amounts of antibody in supernatants. The target monoclonal antibody is also preferably obtained from ascitic fluid derived from a mouse, etc. injected intraperitoneally with live hybridoma cells.

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(b) Purification of the Monoclonal Antibody.

Dike in the separation/purification of conventional polyclonal antibodies, the separation/purification of the anti-G protein coupled receptor monoclonal antibody may be carried out by methods for separating/purifying immunoglobulin (such as salting-out, precipitation with an alcohol, isoelectric precipitation, electrophoresis, adsorption/deadsorption using ion exchangers such as DEAE, ultracentrifugation, gel filtration, specific purifying methods in which only an antibody is collected by treatment with an active adsorbent (such as an antigen-binding solid

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phase, protein  ${\tt A}$  or protein  ${\tt G}$ ) and the bond is dissociated whereupon the antibody is obtained.

The G protein coupled receptor antibody of the present invention which is manufactured by the aforementioned method (a) or (b) is capable of specifically recognizing G protein coupled receptors and, accordingly, it can be used for a quantitative determination of the G protein coupled receptor in test liquid samples and particularly for a quantitative determination by sandwich immunoassays.

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- Thus, the present invention provides, for example, the following methods:
- (i) a quantitative determination of a G protein coupled receptor in a test liquid sample, which comprises(a) competitively reacting the test liquid sample and a
- (a) competitively reacting the test rights sumptoned to labeled G protein coupled receptor with an antibody which reacts with the G protein coupled receptor of the present invention, and
- (b) measuring the ratio of the labeled G protein coupled receptor binding with said antibody; and
- (ii) a quantitative determination of a G protein coupled receptor in a test liquid sample, which comprises
  (a) reacting the test liquid sample with an antibody immobilized on an insoluble carrier and a labeled antibody simultaneously or continuously, and
- 25 (b) measuring the activity of the labeling agent on the insoluble carrier

wherein one antibody is capable of recognizing the N-terminal region of the G protein coupled receptor while another antibody is capable of recognizing the C-terminal region of

30 the G protein coupled receptor.

When the monoclonal antibody of the present invention recognizing a G protein coupled receptor (hereinafter, may be referred to as "anti-G protein coupled receptor antibody") is used, G protein coupled receptors can be measured and,

moreover, can be detected by means of a tissue staining, etc. as well. For such an object, antibody molecules  $\frac{\text{per se}}{\text{may}}$  be used or  $\text{F(ab')}_2$ , Fab' or Fab fractions of the antibody

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molecule may be used too. There is no particular limitation

invention and any measuring method may be used so far as it

for the measuring method using the antibody of the present

relates to a method in which the amount of antibody, antigen or antibody-antigen complex, depending on or corresponding to the amount of antigen (e.g. the amount of G protein coupled S

detected by a chemical or a physical means and then calculated

using a standard curve prepared by a standard solution

containing the known amount of antigen. For example,

receptor, etc.) in the liquid sample to be measured, is

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sandwich method are suitably used and, in terms of sensitivity

nephrometry, competitive method, immunometric method and

and specificity, the sandwich method which will be described

herein later is particularly preferred.

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Examples of the labeling agent used in the measuring

enzymes, fluorescent substances, luminescent substances,

the radioisotope are [  $^{125}$ I], [  $^{13}$ I], [  $^{3}$ H] and [  $^{14}$ C]; preferred examples of the enzyme are those which are

colloids, magnetic substances, etc. Examples of

method using the labeling substance are radioisotopes,

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eta -galactosidase, eta -glucosidase, alkali phosphatase,

stable and with big specific activity, such as

peroxidase and malate dehydrogenase; examples of the fluorescent substance are fluorescamine, fluorescein

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Examples of the carrier are insoluble polysaccharides such as immobilization of proteins or enzymes may be used as well. agarose, dextran and cellulose; synthetic resins such as binding which is usually used for insolubilization or

In a sandwich (or two-site) method, the test liguid is made to react with an insolubilized anti-G protein coupled

polystyrene, polyacrylamide and silicone; glass; etc.

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determined. The first reaction and the second reaction may be of improving the measuring sensitivity, etc., a mixture of two the solid phase is one type or one species but, with an object conducted reversely or simultaneously or they may be conducted react with a labeled anti-G protein coupled receptor antibody (the second reaction) and the activity of the labeling agent on the insoluble carrier is measured whereupon the amount of those mentioned already herein. In the immunoassay by means method of insolubilization (immobilization) may be the same antibody used for the labeled antibody and the antibody for receptor antibody (the first reaction), then it is made to with an interval. The type of the labeling agent and the of a sandwich method, it is not always necessary that the the G protein coupled receptor in the test liguid can be or more antibodies may be used too.

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different each other. Thus, the antibodies used in the first the preferred anti-G protein coupled receptor antibodies used for the first and the second reactions are antibodies wherein and the second reactions are those wherein, when the antibody used in the second reaction recognizes the C-terminal region recognizing the N-terminal region, is preferably used in the their sites binding to the G protein coupled receptors are receptors by the sandwich method of the present invention, recognizing the site other than C-terminal regions, e.g. In the method of measuring G protein coupled of the G protein coupled receptor, then the antibody first reaction.

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or antibodies, a physical adsorption may be used or a chemical

In an insolubilization (immobilization) of antigens

used for binding an antibody or antigen with a labeling agent.

lucigenin, etc. Further, a biotin-avidin system may also be

substance are luminol, luminol derivatives, luciferin,

isothiocyanate, etc.; and examples of the luminescent

present invention may be used in a measuring system other than The anti-G protein coupled receptor antibody of the antigen are made to react with an antibody in a competitive manner, then an unreacted labeled antigen (F) and a labeled B/F separation) and the labeled amount of any of B and F is antigen binding with an antibody (B) are separated (i.e. immunometric method and a nephrometry. In a competitive measured whereupon the amount of the antigen in the test method, an antigen in the test solution and a labeled the sandwich method such as a competitive method, an

solution is determined. With respect to a method for such a reaction, there are a liquid phase method in which a soluble antibody is used as the antibody and the B/F separation is conducted by polyethylene glycol, a second antibody to the above-mentioned antibody, etc.; and a solid phase method in which an immobilized antibody is used as the first antibody or a soluble antibody is used as the first antibody while an immobilized antibody is used as the second antibody.

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In an immunometric method, an antigen in the test solution and an immunometric method, an antigen in the test solution and an immobilized antigen are subjected to a competitive reaction with a certain amount of a labeled antibody followed by separating into solid and liquid phases; or the antigen in the test solution and an excess amount of labeled antibody are made to react, then a immobilized antigen is added to bind an unreacted labeled antibody with the solid phase and separated into solid and liquid phases. After that, the labeled amount of any of the phases is measured to determine the antigen amount in the test solution.

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In a nephrometry, the amount of insoluble sediment which is produced as a result of the antigen-antibody reaction in a gel or in a solution is measured. Even when the antigen amount in the test solution is small and only a small amount of the sediment is obtained, a laser nephrometry wherein scattering of laser is utilized can be suitably used.

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In applying each of those immunological measuring methods (immunoassays) to the measuring method of the present invention, it is not necessary to set up any special condition, operation, etc. therefor. A measuring system (assay system) for G protein coupled receptor may be constructed taking the technical consideration of the persons skilled in the art into consideration in the conventional conditions and operations for each of the methods. With details of those conventional technical means, a variety of reviews, reference books, etc. may be referred to. They are, for example, Hiroshi Irie (ed): "Radioimmunoassay" (Kodansha, Japan, 1974); Hiroshi Irie (ed): "Radioimmunoassay; Second Series" (Kodansha, Japan, 1979); Eiji Ishikwa et al. (ed): "Enzyme Immunoassay" (Igaku Shoin,

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Japan, 1978); Eiji Ishikawa et al. (ed): "Enzyme Immunoassay"

(Second Edition) (Igaku Shoin, Japan, 1982); Eiji Ishikawa

et al. (ed): "Enzyme Immunoassay" (Third Edition) (Igaku Shoin,

Japan, 1987); "Methods in Enzymology" Vol. 70 (Immunochemical

Techniques (Part A)); ibid. Vol. 73 (Immunochemical Techniques

(Part B)); ibid. Vol. 74 (Immunochemical Techniques (Part C));

ibid. Vol. 84 (Immunochemical Techniques (Part D: Selected

Immunoassays)); ibid. Vol. 92 (Immunochemical Techniques

(Part E: Monoclonal Antibodies and General Immunoassay

Methods)); ibid. Vol. 121 (Immunochemical Techniques (Part I:

(7) Preparation of Animals Having the G Protein Coupled Receptor Protein-Encoding DNA of the Present Invention.

Hybridoma Technology and Monoclonal Antibodies)) (Academic

Press); etc.

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It is possible to prepare transgenic animals expressing G protein coupled receptors using G protein coupled receptor protein-encoding DNA. Examples of the animals are warm-blooded mammals such as rats, rabbit, sheep, swines, cattle, cats, dogs and monkeys.

In transferring the G protein coupled receptor protein-encoding DNA to the aimed animal, it is generally advantageous that said DAN is used by ligating with a site at the downstream of a promoter which is capable of expressing in animal cells. For example, when G protein coupled receptor protein DNA is to be transferred to a rabbit, a gene construct ligated with a site at the downstream of various promoters which are capable of expressing the G protein coupled receptor protein DNA derived from an animal compatible to the animal in animal host cells is subjected to a microinjection to the fertilized ovum (oosperm) of the aimed animal (e.g. fertilized ovum (embryo) of rabbit) whereupon the transgenic animal which produces the G protein coupled receptor protein in a high amount can be prepared.

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Examples of the promoters used are promoters derived from virus and ubiquitous expression promoters such as metallothionain promoters may be used but, preferably,

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enolase gene promoters and NGF gene promoters capable of specifically expressing in brain are used.

Transfer of the G protein coupled receptor protein DNA at a fertilized ovum cell stage is secured in order that the DNA can be present in all of embryonal cells and body somatic cells of an aimed animal. The fact that the G protein coupled receptor protein is present in the fertilized ovum cells of the produced transgenic animal after the DNA transfer means that all progeny of the produced transgenic animal have the G protein coupled receptor protein in all of their embryonal cells and somatic cells. Descendants (offsprings) of the animal of this type which inherited the gene have the G protein coupled receptor protein in all of their embryonal cells and somatic cells.

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The transgenic animal to which the G protein coupled receptor protein DNA is transferred can be subjected to a mating and a breeding for generations under a common breeding circumstance as the animal holding said DNA after confirming that the gene can be stably retained. Moreover, male and female animals having the desired DNA are mated to give a homozygote having the transduced gene in both homologous chromosomes and then those male and female animals are mated whereby it is possible to breed for generations so that all descendants have said DNA.

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The animal to which the G protein coupled receptor protein DNA is transferred highly expresses the G protein coupled receptor protein and, accordingly, it is useful as the animal for screening for an agonist or an antagonist to said G protein coupled receptor protein.

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The DNA-transferred animal can be used as a cell source for a tissue culture. For example, DNA or RNA in the tissue of the DNA-transferred mouse is directly analyzed or protein tissues expressed by gene are analyzed whereupon the G protein coupled receptor protein can be analyzed.

Cells of the G protein coupled receptor protein-containing tissue are cultured by standard tissue culture techniques whereupon it is possible to study the function of the cells

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which are usually difficult to culture (e.g. those derived from brain and peripheral tissues) using the resulting culture. By using said cells, it is also possible to select the pharmaceuticals which can potentiate, for example, the functions of various tissues. Moreover, if a cell strain with a high expression is available, it is possible to separate and purify G protein coupled receptor proteins therefrom.

As such, the amount of G protein coupled receptor proteins can now be determined with a high precision using the anti-G protein coupled receptor antibody of the present invention

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(8) Antisense Oligonucleotides Capable of Inhibiting Replication of G Protein Coupled Receptor Protein Gene In another aspect of the present invention, antisense oligonucleotides (nucleic acids) capable of inhibiting the replication or expression of G protein coupled receptor protein gene may be designed and synthesized based on information on the nucleotide sequences of cloned and determined G protein coupled receptor protein-encoding DNAs. Such an antisense oligonucleotide (nucleic acid) is capable of hybridizing with RNA of G protein coupled receptor protein genes to inhibit the synthesis or function of said RNA or of modulating the expression of a G protein coupled receptor protein gene via interaction with G protein coupled receptor protein-related interaction with G protein coupled receptor protein-related

RNA. Oligonucleotides complementary to, and specifically hybridizable with, selected sequences of G protein coupled receptor protein-related RNA are useful in controlling or modulating the expression of a G protein coupled receptor protein gene in vitro and in vivo, and in treating or diagnosing disease states of suspected animals. The term "corresponding" means homologous to or complementary to a particular sequence of the nucleotide sequence or nucleic acid including the gene. As between nucleotides (nucleic acids) peptides (proteins), "corresponding" usually refers to amino acids of a peptide (protein) in an order derived from the

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sequence of a nucleotides (nucleic acids) or its complement.

region, ORF translation initiation codon, 3' untranslated polypeptide translation initiation codon, protein coding loop, 5' end 6-base-pair repeats, 5' end untranslated region, The G protein coupled receptor protein gene 5' end hairpin

complementary to at least a portion of the target, specifically region, 3' end palindrome region, and 3' end hairpin loop may The antisense oligonucleotides may be polydeoxynucleotides hybridizable with the target, is denoted as "antisense". The relationship between the target and oligonucleotides a target among G protein coupled receptor protein genes. be selected as preferred targets though any region may be

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allows for base pairing and base stacking such as is found commercially available) or nonstandard linkages, providing that and synthetic sequence-specific nucleic acid polymers containing nonnucleotide backbones (e.g., protein nucleic acids N-glycoside of a purine or pyrimidine base, or other polymers D-ribose, any other type of polynucleotide which is an the polymers contain nucleotides in a configuration which

containing 2-deoxy-D-ribose, polyribonucleotides containing

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20 DNA and RNA. They may include double- and single-stranded DNA skilled in the art, "caps", methylation, substitution of one or modifications, for example, labels which are known to those and also include, as well as unmodified forms of the as well as double- and single-stranded RNA and DNA:RNA hybrids, polynucleotide or oligonucleotide, known types of

more of the naturally occurring nucleotides with analogue, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates,

phosphotriesters, phosphoramidates, carbamates, etc.) and with phosphorothioates, phosphorodithioates, etc.), those containing charged linkages or sulfur-containing linkages (e.g., peptides, poly-L-lysine, etc.) and saccharides (e.g., nucleases, nuclease inhibitors, toxins, antibodies, signal pendant moieties, such as, for example, proteins (including

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metals, radioactive metals, boron, oxidative metals, etc.), acridine, psoralen, etc.), those containing chelators (e.g., monosaccharides, etc.), those with intercalators (e.g.,

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include modifications on the sugar moiety, e.g., wherein one or heterocycles. Modified nucleosides or nucleotides will also and pyrimidines, acylated purines and pyrimidines, or other been modified. Such modifications include methylated purines pyrimidine bases, but also other heterocyclic bases which have (e.g., alpha anomeric nucleic acids, etc.). The terms those containing alkylators, those with modified linkages those moieties which contain not only the known purine and "nucleoside", "nucleotide" and "nucleic acid" will include

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10 aliphatic groups, or are functionalized as ethers, amines, or more of the hydroxyl groups are replaced with halogen,

15 nucleic acid are, but not limited to, degradation-resistant invention are modifications that are designed to: modifications of the antisense nucleic acids of the present poly- or oligonucleoside amides. Preferred design sulfurized and thiophosphate derivatives of nucleic acids, and is RNA, DNA or a modified nucleic acid. Examples of modified The antisense nucleic acid of the present invention

20 increase the intracellular stability of the nucleic acid;

increase the cellular permeability of the nucleic acid;

(3) increase the affinity of the nucleic acid for the target sense strand; or

(4) decrease the toxicity (if any) of the nucleic acid.

25 pp.247, 1992; Vol. 8, pp.395, 1992; S. T. Crooke et al. ed., as described in J. Kawakami et al., Pharm Tech Japan, Vol. 8, Many such modifications are known to those skilled in the art, The nucleic acids may contain altered or modified sugars, bases Antisense Research and Applications, CRC Press, 1993; etc.

30 or linkages, be delivered in specialized systems such as attached moieties. Such attached moieties include polycationic moieties such as polylysine that act as charge neutralizers of the phosphate backbone, or hydrophobic moieties such as lipids liposomes, microspheres or by gene therapy, or may have

35 (e.g., phospholipids, cholesterols, etc.) that enhance nucleic acid. Preferred lipids that may attached are interaction with cell membranes or increase uptake of the

protecting groups known to those skilled in the art, including glycols such as polyethylene glycols, tetraethylene glycol and attached at the 3' or 5' ends of the nucleic acids, and also linkage. Other moieties may be capping groups specifically placed at the 3' or 5' ends of the nucleic acids to prevent may be attached through a base, sugar, or internucleoside degradation by nuclease such as exonuclease, RNase, etc. capping groups include, but are not limited to, hydroxyl chloroformate, cholic acid, etc.). The moieties may be cholesterols or derivatives thereof (e.g., cholesteryl the like.

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proteins. The nucleic acid can be placed in the cell through The inhibitory activity of antisense nucleic acids and in vivo translation system of G protein coupled receptor can be examined using the transformant (or transfectant) of expression system of the present invention, or the in vitro the present invention, the in vitro and in vivo gene any number of ways known per se.

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amino acids and so forth are those recommended by the IUPAC-IUB Amino acids for which optical isomerism is possible are, unless Commission on Biochemical Nomenclature or those conventionally application, the abbreviations used for bases (nucleotides), In the specification and drawings of the present used in the art. Examples thereof are given below. otherwise specified, in the L form. 20 25

DNA : Deoxyribonucleic acid

CDNA: Complementary deoxyribonucleic acid

: Adenine

: Thymine

: Guanine

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: Cytosine

RNA: Ribonucleic acid

mRNA : Messenger ribonucleic acid

dATP: Deoxyadenosine triphosphate

dTTP: Deoxythymidine triphosphate 35

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dGTP: Deoxyguanosine triphosphate dCTP: Deoxycytidine triphosphate

ATP : Adenosine triphosphate

EDTA: Ethylenediamine tetraacetic acid

SDS : Sodium dodecyl sulfate

EIA: Enzyme Immunoassay

Glycine (or Glycyl) G, Gly:

Alanine (or Alanyl) Valine (or Valyl) A, Ala: V, Val:

Leucine (or Leucyl) L, Leu:

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Isoleucine (or Isoleucyl) I, Ile:

Serine (or Seryl) Ser:

Thr: Ë

Threonine (or Threonyl)

Cysteine (or Cysteinyl) C, Cys: Methionine (or Methionyl) M, Met:

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Glutamic acid (or Glutamy1) E, Glu:

Aspartic acid (or Aspartyl) D, Asp:

Lysine (or Lysyl) K, Lys:

Histidine (or Histidyl) Arginine (or Arginyl') R, Arg: H, His:

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Pheylalanine (or Pheylalanyl) F, Phe:

Tyrossine (or Tyrosyl) Y, Tyr: Tryptophan (or Tryptophanyl) W, Trp:

Proline (or Prolyl) P, Pro:

Asparagine (or Asparaginyl) N, Asn:

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Glutamine (or Glutaminyl) Q, Gln:

Norvaline (or Norvalyl) NVal:

Pyroglutamic acid (or Pyroglutamyl) pgln:

7 -Butyrolacton-7 -carbonyl Blc:

2-Ketopiperidiny1-6-carbonyl Kpc:

Methy]

Otc:

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3-Oxoperhydro-1,4-thiazin-5-carbonyl

Ethy 1

Buty1

Pheny1

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Thiazolidinyl-4(R)-carboxamide

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Number IFO 15747.

The transformant Escherichia coli, designated JM109/p3H2-34, which is obtained in the Example 8 mentioned herein below, is on deposit under the terms of the Budapest Treaty from October 12, 1994, with NIBH and has been assigned the Accession Number FERM BP-4828. It is also on deposit from October 12, 1994 with IFO and has been assigned the Accession Number IFO 15749.

The transformant Escherichia coli, designated

JM109/pMD4, which is obtained in the Example 9 mentioned herein
below, is on deposit under the terms of the Budapest Treaty
from November 11, 1994, with NIBH and has been assigned the
Accession Number FERM BP-4888. It is also on deposit from
November 17, 1994 with IFO and has been assigned the Accession
Number IFO 15765.

The transformant Escherichia coli, designated JM109/pMGR20, which is obtained in the Example 10 mentioned herein below, is on deposit under the terms of the Budapest Treaty from December 15, 1994, with NIBH and has been assigned the Accession Number FERM BP-4937. It is also on deposit from December 14, 1994 with IFO and has been assigned the Accession Number IFO 15773.

The transformant Escherichia coli, designated JM109/pMJ10, which is obtained in the Example 12 mentioned to herein below, is on deposit under the terms of the Budapest Treaty from December 15, 1994, with NIBH and has been assigned the Accession Number FERM BP-4936. It is also on deposit from December 16, 1994 with IFO and has been assigned the Accession Number IFO 15784.

The transformant Escherichia coli, designated JM109/pMH28, which is obtained in the Example 14 mentioned herein below, is on deposit under the terms of the Budapest Treaty from January 13, 1995, with NIBH and has been assigned the Accession Number FERM BP-4970. It is also on deposit from January 20, 1995 with IFO and has been assigned the Accession Number IFO 15791.

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The transformant Escherichia coli, designated

Science and Technology, Ministry of International Trade and Bioscience and Human-Technology (NIBH), Agency of Industrial herein below, is on deposit under the terms of the Budapest INVa F'/p19P2, which is obtained in the Example 3 mentioned the Accession Number FERM BP-4775. It is also on deposit from Treaty from August 9, 1994, with NIBH and has been assigned assigned the Accession Number IFO 15739. the Institute for Fermentation, Osaka, Japan (IFO) and has been FERM BP-4776. It is also on deposit from August 22, 1994 with Industry, Japan and has been assigned the Accession Number Treaty from August 9, 1994, with the National Institute of Number IFO 15740. August 22, 1994 with IFO and has been assigned the Accession herein below, is on deposit under the terms of the Budapest INVa F'/pG3-2, which is obtained in the Example 4 mentioned The transformant Escherichia coli, designated The transformant Escherichia coli, designated

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The transformant Escherichia coli, designated INVa F'/p63A2, which is obtained in the Example 5 mentioned herein below, is on deposit under the terms of the Budapest Treaty from August 9, 1994, with NIBH and has been assigned the Accession Number FERM BP-4777. It is also on deposit from August 22, 1994 with IFO and has been assigned the Accession Number IFO 15738.

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The transformant Escherichia coli, designated JM109/phGR3, which is obtained in the Example 6 mentioned herein below, is on deposit under the terms of the Budapest Treaty from September 27, 1994, with NIBH and has been assigned the Accession Number FERM BP-4807. It is also on deposit from September 22, 1994 with IFO and has been assigned the Accession Number IFO 15748.

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The transformant Escherichia coli, designated JM109/p3H2-17, which is obtained in the Example 7 mentioned herein below, is on deposit under the terms of the Budapest Treaty from September 27, 1994, with NIBH and has been assigned the Accession Number FERM BP-4806. It is also on deposit from September 22, 1994 with IFO and has been assigned the Accession

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JM109/pMN7, which is obtained in the Example 16 mentioned herein below, is on deposit under the terms of the Budapest Treaty from February 22, 1995, with NIBH and has been assigned the Accession Number FERM BP-5011. It is also on deposit from February 27, 1995 with IFO and has been assigned the Accession Number IFO 15803.

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The transformant Escherichia coli, designated JM109/p5S38, which is obtained in the Example 17 mentioned herein below, is on deposit under the terms of the Budapest Treaty from October 27, 1994, with NIBH and has been assigned the Accession Number FERM BP-4856. It is also on deposit from October 25, 1994 with IFO and has been assigned the Accession Number IFO 15754.

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The transformant Escherichia coli, designated JM109/pMAH2-17, which is obtained in the Example 19 mentioned herein below, is on deposit under the terms of the Budapest Treaty from April 7, 1995, with NIBH and has been assigned the Accession Number FERM BP-5073. It is also on deposit from March 31, 1995 with IFO and has been assigned the Accession Number IFO 15813.

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The transformant Escherichia coli, designated JM109/pWN128, which is obtained in the Example 20 mentioned herein below, is on deposit under the terms of the Budapest Treaty from March 17, 1995, with NIBH and has been assigned the Accession Number FERM BP-5039. It is also on deposit from March 22, 1995 with IFO and has been assigned the Accession Number IFO 15810.

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The transformant Escherichia coli, designated JM109/phAH2-17, which is obtained in the Example 21 mentioned herein below, is on deposit under the terms of the Budapest Treaty from July 20, 1995, with NIBH and has been assigned the Accession Number FERM BP-5168. It is also on deposit from July 14, 1995 with IFO and has been assigned the Accession Number IFO 15856.

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Each SEQ ID NO set forth in the SEQUENCE LISTING of the specification refers to the following sequence:

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[SEQ ID NO: 24] is a partial amino acid sequence of the human pituitary gland-derived G protein coupled receptor protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2,

[SEQ ID NO: 25] is a partial amino acid sequence of the human pituitary gland-derived G protein coupled receptor protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p1992,

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[SEQ ID NO: 26] is an entire amino acid sequence of the humark, pituitary gland-derived G protein coupled receptor protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in phGR3,

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(SEQ ID NO: 27) is a partial amino acid sequence of the mouse pancreatic  $\beta$  -cell line, MIN6-derived G protein coupled

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receptor protein encoded by the mouse pancreatic  $\beta$  -cell line, MIN6-derived G protein coupled receptor protein cDNA fragment having a nucleotide sequence (SEQ ID NO: 32), derived based upon the nucleotide sequences of the mouse pancreatic  $\beta$  -cell line, MIN6-derived G protein coupled receptor protein cDNA fragments each included in pG3-2 and pG1-10,

[SEQ ID NO: 28] is a partial amino acid sequence of the mouse pancreatic  $\beta$  -cell line, MIN6-derived G protein coupled receptor protein encoded by p5s38,

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[SEQ ID NO: 29] is a nucleotide sequence of the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p1992,

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[SEQ ID NO: 30] is a nucleotide sequence of the human pituitary gland-derived G protein coupled receptor protein CDNA fragment included in p19P2,

[SEQ ID NO: 31] is an entire nucleotide sequence of the human pituitary gland-derived G protein coupled receptor protein CDNA included in phGR3,

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[SEQ ID NO: 32] is a nucleotide sequence of the mouse pancreatic  $\beta$  -cell line, MIN6-derived G protein coupled receptor protein cDNA, derived based upon the nucleotide sequences of the mouse pancreatic  $\beta$  -cell line, MIN6-derived G protein coupled receptor protein cDNA fragments each

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included in pG3-2 and pG1-10, [SEQ ID NO: 33] is a nucleotide sequence of the mouse pancreatic  $\beta$  -cell line, MIN6-derived G protein cDNA included in pGC38

5 [SEQ ID NO: 34] is a partial amino acid sequence of the human amygdaloid nucleus-derived G protein coupled receptor protein encoded by the cDNA fragment included in p63A2,

(SEQ ID NO: 35] is a partial amino acid sequence of the human amygdaloid nucleus-derived G protein coupled receptor protein encoded by the cDNA fragment included in p63A2,

[SEQ ID NO: 36] is a nucleotide sequence of the human amygdaloid nucleus-derived G protein coupled receptor protein cDNA fragment included in p63A2,

(SEQ ID NO: 37) is a nucleotide sequence of the human
15 amygdaloid nucleus-derived G protein coupled receptor protein
cDNA fragment included in p63A2,

[SEQ ID NO: 38] is a partial amino acid sequence encoded by the mouse pancreatic  $\beta$  -cell line, MIN6-derived G protein coupled receptor protein cDNA included in p3H2-17,

20 [SEQ ID NO: 39] is a full-length amino acid sequence encoded by the open reading frame of the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein cDNA included in pMAH2-17,

[SEQ ID NO: 40] is a nucleotide sequence of the mouse pancreatic  $\beta$  -cell line, MIN6-derived G protein coupled receptor protein cDNA included in p3H2-17,

(SEQ ID NO: 41) is a nucleotide sequence of the mouse pancreatic  $\beta$  -cell line, MIN6-derived G protein coupled receptor protein cDNA included in pMAH2-17,

30 (SEQ ID NO: 42) is a partial amino acid sequence encoded by the mouse pancreatic \(\theta\) -cell line, MIN6-derived G protein coupled receptor protein cDNA included in p3H2-34,

[SEQ ID NO: 43] is a nucleotide sequence of the mouse

pancreatic  $\theta$  -cell line, MIN6-derived G protein coupled receptor protein cDNA fragment included in p3H2-34,

(SEQ ID NO: 44] is a partial amino acid sequence encoded by the rabbit gastropyrolic part smooth muscle-derived G

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protein coupled receptor protein cDNA included in pMD4, [SEQ ID NO: 45] is a nucleotide sequence of the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in pMD4,

5 [SEQ ID NO: 46] is an entire amino acid sequence encoded by the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein cDNA included in pMGR20, [SEQ ID NO: 47] is a nucleotide sequence of the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein cDNA included in pMGR20,

[SEQ ID NO: 48] is a partial amino acid sequence encoded by the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA included in pMJ10, [SEQ ID NO: 49] is a nucleotide sequence of the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in pMJ10, [SEQ ID NO: 50] is a partial amino acid sequence encoded by the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA included in pMH28,

20 [SEQ ID NO: 51] is a nucleotide sequence of the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in pMH28, [SEQ ID NO: 52] is a partial amino acid sequence encoded by the rabbit gastropyrolic part smooth muscle-derived G protein coupled receptor protein cDNA included in pMN7,

protein coupled receptor protein cDNA included in pMN7,

[SEQ ID NO: 53] is a nucleotide sequence of the rabbit
gastropyrolic part smooth muscle-derived G protein coupled
receptor protein cDNA fragment included in pMN7,

[SEQ ID NO: 54] is a partial amino acid sequence encoded
by the rabbit gastropyrolic part smooth muscle-derived G
protein coupled receptor protein cDNA included in pMN128,

[SEQ ID NO: 55] is a nucleotide sequence of the rabbit
gastropyrolic part smooth muscle-derived G protein coupled
receptor protein cDNA fragment included in pMN128,

SEQ ID NO: 56 is a full-length amino acid sequence of the human-derived G protein coupled receptor protein encoded by the human-derived G protein coupled receptor protein cDNA

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included in phAH2-17, and

[SEQ ID NO: 57] is a nucleotide sequence of the human-derived G protein coupled receptor protein cDNA included in phAH2-17.

#### EXAMPLES

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Described below are working examples of the present invention which are provided only for illustrative purposes, and not to limit the scope of the present invention. In light of the present disclosure, numerous embodiments within the scope of the claims will be apparent to those of ordinary skill in the art.

### Example 1

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Preparation of Synthetic DNA Primer for Amplifying DNA Coding for G Protein Coupled Receptor Protein

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(U00442, U00442), human-derived neuromedin B receptor protein RANTES receptor protein (L10918, HUMRANTES), human Burkitt's human-derived  $C_{\mathsf{S}}$ a receptor protein (HUMC5AAR), human-derived coding for the known amino acid sequences corresponding to (L14856, HUMSOMAT), rat-derived  $\mu$  -opioid receptor protein (U02083, RNU02083), rat-derived  $\kappa$  -opioid receptor protein human-derived TRH receptor protein (HTRHR), human-derived lymphoma-derived unknown ligand receptor protein (X68149, M73482, HUMNMBR), human-derived muscarinic acetylcholine unknown ligand receptor protein (HUMRDCIA), human-derived receptor protein (X15266, HSHM4), rat-derived adrenaline 2, B receptor protein (L08609, RATAADRE01), human-derived unknown ligand receptor protein (M84605, HUMOPIODRE) and A comparison of deoxyribonucleotide sequences HSBLR1A), human-derived somatostatin receptor protein rat-derived adrenaline  $a_2^{}\mathrm{B}$  receptor protein (M91466, somatostatin 3 receptor protein (M96738, HUMSSTR3X), RATA2BAR) was made. As a result, highly homologous or near.the first membrane-spanning domain each of regions or parts were found (Figure 1).

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Further, a comparison of deoxynucleotide sequences coding for the known amino acid sequences corresponding to or near the sixth membrane-spanning domain each of mouse-derived

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unknown ligand receptor protein (M80481, MUSGIR), human-derived bombesin receptor protein (L08893, HUMBOMB3S), human-derived adenosine A2 receptor protein (S46950, S46950), mouse-derived unknown ligand receptor protein (D21061, MUSGPCR), mouse-derived adenosine A1 receptor protein (J05189, RATAIBARA), rat-derived neuromedin K receptor protein (M69045, RATAIBARA), human-derived adenosine A1 receptor protein (M69045, RATAIBARA), human-derived adenosine A3 receptor protein (M67414, HUMNEKAR), rat-derived neurokinin A receptor protein (M94152, RATAIBARA), human-derived neurokinin 3 receptor protein (S86390, S86371S4), rat-derived unknown ligand receptor protein (S61496, RNCGPCR), human-derived somatostatin 4 receptor protein (L07061, HUMSSTR42) and rat-derived GNRH receptor protein (L07061, HUMSSTR42) and rat-derived GNRH receptor protein (M31670, RATGNRHA) was made. As a result, highly homologous

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The aforementioned abbreviations in the parentheses are identifiers (reference numbers) which are indicated when GenBank/EMBL Data Bank is retrieved by using DNASIS Gene/Protein Sequencing Data Base (CD019, Hitachi Software Engineering, Japan) and are usually called "Accession Numbers" or "Entry Names". HTRHR is, however, the sequence as disclosed in Japanese Unexamined Patent Publication No. 286986/1993 (EPA 638645).

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regions or parts were found (Figure 2).

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Specifically, it was planned to incorporate mixed bases relying upon the base regions that were in agreement with cDNAs coding for a large number of receptor proteins in order to enhance base agreement of sequences with as many receptor cDNAs as possible even in other regions. Based upon these sequences, the degenerate synthetic DNA having a nucleotide sequence represented by SEQ ID NO: 1 which is complementary to the homologous nucleotide sequence represented by SEQ ID NO: 2 which is complementary to the homologous nucleotide sequence represented by SEQ ID NO: 2 which is complementary to the homologous nucleotide sequence of Figure 2 were produced. Nucleotide synthesis was carried out by a DNA synthesizer.

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5'-CGTGG (G or C) C (A or C) T (G or C) (G or C) TGGGCAAC [Synthetic DNAs] (A, G, C or T) (C or T) CCTG-3' SEQ ID NO: 1)

Ų 5'-GT (A, G, C or T) G (A or T) (A or G) (A or G) GGCA (A, G, C or T) CCAGCAGA (G or T) GGCAAA-3'

(SEQ ID NO: 2)

plurality of bases, leading to multiple oligonucleotides in the presence of a mixture of plural bases at the time of synthesis. parentheses of the aforementioned DNAs were incorporated in the primer preparation. In other words, nucleotide residues in The parentheses indicate the incorporation of a

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15 Somatostatin Receptor Protein-Encoding DNA Human D5 Dopamine Receptor Protein-Encoding DNA, and Rat Isolation of Human Somatostatin Receptor Protein-Encoding DNA, Amplification of DNA by Polymerase Chain Reaction (PCR) cDNAs (QuickClone, CLONTECH Laboratories, Inc.)

2.5 units of Tag DNA polymerase (Takara Shuzo Co., Japan) of 1 $\mu$  M, 2.5 mM dNTPs (deoxyribonucleoside triphosphates), and synthetic DNA primers prepared in Example 1 each in an amount gland and rat brain each in an amount of 1 ng as templates, the prepared from human brain amygdaloid nucleus, human pituitary were mixed together with a buffer attached to the enzyme kit

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such that the total amount was 100  $\mu$  l. The polymerase chain Amplification of DNAs was confirmed by 1.2% agarose this one cycle was repeated 30 times to amplify DNAs. 30 sec., 45 °C for 1 min. and 60 °C for 3 min.. Totally by Perkin-Elmer Co. One cycle was set to include 96 °C for reaction was carried out by using a Thermal Cycler manufactured

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electrophoresis [Figure 17].

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The DNA was transfected into E. coli attached to the amplified by the PCR was inserted into a plasmid vector, pCR  $^{
m TH}$  II. Isolation of Amplified DNA and Analysis of DNA Sequence By using a TA Cloning Kit (Invitrogen Co.), the DNA

10 S kit to form an amplified DNA library. Colonies formed by the an automatic plasmid extracting machine (Kurabo Co., Japan). are inserted. They were cultured in an LB culture medium to order to separate only white colonies in which DNA fragments indolyl- $\beta$  -D-galactoside)-added LB (Luria-Bertani) plates in activity of eta -galactosidase on X-gal (5-bromo-4-chloro-3transformants were selected under guidance based on the which ampicillin was added and plasmid DNAs were prepared with An aliquot of the DNA thus prepared was further

5 20 marker. An aliquot of the plasmid DNA thus prepared was inserted, and a DNA yield each of clones was compared with a digested with EcoRI to confirm DNA fragments that were seguencing kit (Applied Biosystems Co.). precipitated in ethanol, and the resulting product was then treated with RNase, extracted with phenol/chloroform, reacted for sequencing by using a DyeDeoxy terminator cycle

25 The nucleotide sequences obtained are shown in Figures 18, 19, analyzed by using DNASIS (Hitachi Software Engineering, Japan). Biosystems Co. The nucleotide sequences obtained were fluorescent automatic sequencer manufactured by Applied Sequencing was carried out by using a 370A

retrieval, it was learned that the DNAs obtained were somatostatin receptor protein [Figure 21] that can be and 19], human D5 dopamine receptor protein [Figure 20] and rat DNAs encoding human somatostatin receptor protein [Figures 18 20 and 21. From these Figures and the results of homology classified each into a group of G protein coupled receptor

The underlined part represents the 5' side synthetic DNA primer the clone, A58, is a human somatostatin receptor cDNA. sequence encoding somatostatin receptor (HUMSOMAT) and seguence of the DNA is in agreement with the nucleotide In Figure 18 as described herein, the nucleotide

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used for the PCR. Thus, even when parts of the nucleotide seguence are mismatched, amplification is effected to a sufficient degree by the PCR.

It will be understood from Figure 19 that the clone, A58 is in good agreement with the nucleotide sequence coding for the human somatostatin receptor (HUMSOMAT) even when the sequencing is carried out from the opposite side. The underlined part represents the 3' side synthetic DNA primer used for the PCR. In this figure, the nucleotide sequences are mismatched to some extent even in the portions other than the primer portion presumably due to base substitution at the time of PCR or due to partial deviation in the sequencing reaction. It can be confirmed via sequencing of chains complementary thereto as required.

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In Figure 20 as described herein, the nucleotide sequence of the DNA is in good agreement with a nucleotide sequence coding for the human D5 dopamine receptor (HUMDRD5A) except the primer portion (underlined). It was learned that the clone, 57-A-2, is a human D5 dopamine receptor cDNA.

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In Figure 21 as described herein, the DNA is in good agreement with a nucleotide sequence coding for the rat somatostatin receptor (RNU04738) except the primer portion (underlined). It was learned that the clone, B54, is a rat somatostatin receptor cDNA.

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Example

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Isolation of Human Pituitary Gland-Derived G Protein Coupled Receptor Protein-Encoding DNA

(1) Amplification of Receptor cDNA by PCR Using Human Pituitary Gland-Derived cDNA

By using human pituitary gland-derived cDNA (QuickClone, CLONTECH Laboratories, Inc.) as a template, PCR amplification using the DNA primers synthesized in Example 1 was carried out. The composition of the reaction solution consisted of the synthetic DNA primers (SEQ: 5' primer sequence and 3' primer sequence) each in an amount of  $1\mu$  M, 1 ng of the template cDNA, 0.25 mM dNTPs, 1  $\mu$  1 of Tag DNA polymerase and

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a buffer attached to the enzyme kit, and the total amount of the reaction solution was made to be 100 $\mu$  1. The cycle for amplification including 95 °C for 1 min., 55 °C for 1 min. and 72 °C for 1 min. was repeated 30 times by using a Thermal Cycler (Perkin-Elmer Co.). Prior to adding Tag DNA polymerase, the remaining reaction solution was mixed and was heated at 95 °C for 5 minutes and at 65 °C for 5 minutes. The amplified products were confirmed relying upon 1.2% agarose gel electrophoresis and ethidium bromide staining.

(2) Subcloning of PCR Product into Plasmid Vector and Selection of Novel Receptor Candidate Clone via Decoding Nucleotide Sequence of Inserted cDNA Region

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The PCR products were separated by using a 0.8% low-melting temperature agarose gel, the band parts were excised from the gel with a razor blade, and were heat-melted, extracted with phenol and precipitated in ethanol to recover DNAs. According to the protocol attached to a TA Cloning Kit (Invitrogen Co.), the recovered DNAs were subcloned into the plasmid vector, pCR MI (TM represents registered trademark). The recombinant vectors were introduced into E. Coli INVa F' competent cells (Invitrogen Co.) to produce transformants. Then, transformant clones having a cDNA-inserted fragment were selected in an LB agar culture medium containing ampicillin and X-gal. Only transformant clones exhibiting white color were picked with a sterilized toothstick to obtain transformant Escherichia coli INVa F'/p1992.

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The individual clones were cultured overnight in an LB culture medium containing ampicillin and treated with an automatic plasmid extracting machine (Kurabo Co., Japan) to prepare plasmid DNAs. An aliquot of the DNA thus prepared was cut by EcoRI to confirm the size of the cDNA fragment that was inserted. An aliquot of the remaining DNA was further processed with RNase, extracted with phenol/chloroform, and precipitated in ethanol so as to be condensed. Sequencing was carried out by using a Dyebbeoxy terminator cycle sequencing kit (ABI Co.), the DNAs were decoded by using a

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primers [Figures 22 and 23]. portions represent regions corresponding to the synthetic nucleotide sequences obtained were read by using DNASIS (Hitachi System Engineering Co., Japan). The underlined fluorescent automatic sequencer, and the data of the

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the plasmid, p19P2, possessed by the transformant receptor protein was encoded by the cDNA fragment insert in As a result, it was learned that a novel G protein coupled determined nucleotide sequences [Figures 22 and 23]. carried out in view of hydrophobicity plotting (Figures 24 and sequences [Figures 22 and 23], and homology retrieval was Escherichia coli INVa F'/p19P2. To further confirm this the nucleotide sequences were converted into amino acid fact, by using DNASIS (Hitachi System Engineering Co., Japan) relative to neuropeptide Y receptor proteins [Figure 26]. 25] and at the amino acid sequence level to find homology Homology retrieval was carried out based upon the

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# Example 4

Isolation of Mouse Pancreas-Derived G Protein Coupled Receptor

# Protein-Encoding DNA

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 Preparation of Poly(A) RNA Fraction from Mouse Pancreatic  $\beta$  -Cell Strain, MIN6 and Synthesis of cDNA

Vol. 127, No. 1, p.126-132) according to the guanidine (1:1), precipitated in ethanol, and was then dissolved in 30 The reaction product was extracted with phenol/chloroform reverse transcriptase kit to synthesize complementary DNAs. transcriptase (BRL Co.) in the buffer attached to the MMLV to reaction with mouse Moloney Leukemia virus (MMLV) reverse (BRL Co.) as a primer, and the resulting mixture was subjected of the poly(A) RNA fraction was added a random DNA hexamer with a mRNA purifying kit (Pharmacia Co.). Next, to 5  $\mu$  g 181-184 (1979)) and, then, poly(A) RNA fractions were prepared thiocyanate method (Kaplan B.B. et al., Biochem. J., 183,  $extcolor{black}{eta}$  -cell strain, MIN6 (Jun-ichi Miyazaki et al., Endocrinology,  $\mu$  l of TE buffer (10 mM Tris-HCl at pH8.0, 1 mM EDTA at pH8.0). A total RNA was prepared from the mouse pancreatic

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(2) Amplification of Receptor cDNA by PCR Using MIN6-Derived cDNA and Sequencing

10 σ as in Example 3(2). The resulting PCR product was in Example 1 was carried out under the same conditions step (1), PCR amplification using the DNA primers synthesized was transfected into E. coli INVa F' to obtain transformed as in Example 2 to obtain a plasmid, pG3-2. The plasmid pG3-2 subcloned into the plasmid vector, pCR  $^{\text{TM}}$  II, in the same manner Escherichia coli INVa F'/pG3-2. from the mouse pancreatic eta -cell strain, MIN6 in the above By using, as a template, 5  $\mu$  l of cDNA prepared

synthetic primer represented by the following sequence: al., "Science, 244:569-572, 1989", i.e., a degenerate amplification using DNA primers as disclosed in Libert F. et from the mouse pancreatic eta -cell strain, MIN6, PCR By using, as a template, 5  $\mu$  l of the cDNA prepared

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5'-CTGTG (C or T) G (C or T) (G or C) AT (C or T) GCIIT (G or T) GA (C or T) (A or C) G (G or C) TAC-3' (SEQ ID NO: 60)

a degenerate synthetic primer represented by the following wherein I is inosine; and

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5'-A (G or T) G (A or T) AG (A or T) AGGGCAGCCAGCAGAI (G or C) (A or G) (C or T) GAA-3' (SEQ ID NO: 61)

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plasmid vector, pCR  $^{\mathrm{TM}}$ II, in the same manner as described in was carried out under the same conditions as in Working Example 1. The resulting PCR product was subcloned into the wherein I is inosine,

Example 3(2) to obtain a plasmid, pG1-10. (sequencing) was carried out with a DyeDeoxy terminator The reaction for determining the nucleotide sequence

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cycle sequencing kit (ABI Co.), the DNA was decoded with the fluorescent automatic sequencer (ABI Co.), and the data

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of the nucleotide sequence obtained were analyzed with DNASIS (Hitachi System Engineering Co., Japan).

Figure 27 shows a mouse pancreatic  $\beta$  -cell strain MIN6-derived G protein coupled receptor protein-encoding DNA and an amino acid sequence encoded by the isolated DNA based upon the nucleotide sequences of plasmids pG3-2 and pG1-10 which are held by the transformant Escherichia coli INVa F'/pG3-2. The underlined portions represent regions corresponding to the synthetic primers.

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Homology retrieval was carried out based upon the determined nucleotide sequence [Figure 27]. As a result, it was learned that a novel G protein coupled receptor protein was encoded by the cDNA fragment obtained. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the nucleotide sequence was converted into an amino acid sequence [Figure 27], hydrophobicity plotting was carried out to confirm the presence of six hydrophobic regions [Figure 28]. Upon comparing the amino acid sequence with that of p19P2 obtained in Example 3, furthermore,

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a high degree of homology was found as shown in [Figure 61]. As a result, it is strongly suggested that the G protein coupled receptor proteins encoded by pG3-2 and pG1-10 recognize the same ligand as the G protein coupled receptor protein encoded by p19P2 does while the animal species from which the receptor proteins encoded by pG3-2 and pG1-10 are derived is different from that from which the receptor protein encoded by p19P2 is.

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# Example 5

Isolation of Human Amygdaloid Nucleus-Derived G Protein Coupled Receptor Protein-Encoding DNA

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 Amplification of Receptor cDNA by PCR Using Human Amygdaloid Nucleus-Derived cDNA

By using an amplified human amygdala-derived cDNA (QuickClone, CLONTECH Laboratories, Inc.) as a template, PCR amplification using the DNA primers synthesized in Example 1 was carried out. The composition of the reaction solution

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consisted of the synthetic DNA primers (SEQ: 5' primer sequence and 3' primer sequence) each in an amount of 1 $\mu$  M, 1 ng of the template cDNA, 0.25 mM dNTPs, 1  $\mu$  1 of Taq DNA polymerase and a buffer attached to the enzyme kit, and the total amount of the reaction solution was made to be 100 $\mu$  1. The cycle for amplification including 95 °C for 1 min., 55 °C for 1 min. and 72 °C for 1 min. was repeated 30 times by using a Thermal Cycler (Perkin-Elmer Co.). Prior to adding Taq DNA polymerase, the remaining reaction solution was mixed and was heated at 95 °C for 5 minutes. The amplified products were confirmed relying upon 1.2% agarose gel electrophoresis and ethidium bromide staining.

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2) Subcloning of PCR Product into Plasmid Vector and Selection of Novel Receptor Candidate Clone via Decoding Nucleotide Sequence of Inserted cDNA Region

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The PCR products were separated by using a 0.8% low-melting temperature agarose gel, the band parts were excised from the gel with a razor blade, and were heat-melted, extracted with phenol and precipitated in ethanol to recover DNAs. According to the protocol attached to a TA Cloning Kit (Invitrogen Co.), the recovered DNAs were subcloned to the plasmid vector, pCR MI. The recombinant vectors were introduced into E. coli INVa F' competent cells (Invitrogen Co.) to produce transformants. Then, transformant clones having a cDNA-inserted fragment were selected in an LB agar culture medium containing ampicillin and X-gal. Only transformant clones exhibiting white color were picked with a sterilized toothstick to obtain transformant Escherichia

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The individual clones were cultured overnight in an automatic plasmid extracting ampicillin and treated with an automatic plasmid extracting machine (Kurabo Co., Japan) to prepare plasmid DNAs. An aliquot of the DNA thus prepared was cut by EcoRI to confirm the size of the cDNA fragment that was inserted. An aliquot of the remaining DNA was further processed with RNase, extracted with phenol/chloroform, and

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precipitated in ethanol so as to be condensed. Sequencing - 199-

nucleotide sequences obtained were read by using DNASIS sequencing kit (ABI Co.), the DNAs were decoded by using a fluorescent automatic sequencer, and the data of the was carried out by using a DyeDeoxy terminator cycle

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receptor protein was encoded by the cDNA fragment insert in As a result, it was learned that a novel G protein coupled determined nucleotide sequences [Figures 29 and 30]. (Hitachi System Engineering Co., Japan). fact, by using DNASIS (Hitachi System Engineering Co., Japan) Escherichia coli INVa F'/p63A2. To further confirm this the plasmid, p63A2 possessed by the transformant Homology retrieval was carried out based upon the

15 sequences [Figures 29 and 30], and homology retrieval was the nucleotide sequences were converted into amino acid relative to mouse GIR [Figure 33]. 32] and at the amino acid sequence level to find homology carried out in view of hydrophobicity plotting [Figures 31 and

# Example 6

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Cloning of Human Pituitary Gland-Derived G Protein Coupled Receptor Protein cDNA

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(1) Cloning of cDNA Comprising Whole Coding Regions for Receptor Protein from Human Pituitary Gland-Derived **cDNA** Library

A gtll phage vector is used (CLONTECH Laboratories, Inc.) CLH L1139b) was employed as a human pituitary gland-derived CDNA library. The human pituitary gland cDNA library  $(2 \times 10^6 \text{ pfu (plague forming units)})$  was mixed with The DNA library constructed by Clontech Co. wherein

E. coli Y1090 treated with magnesium sulfate, and incubated onto the filter. The filter was denatured with an alkali and on which plaques were formed and the plaque was transferred ampicillin). A nitrocellulose filter was placed on the plate (Pharmacia Co.) LB. The E, coli was plated onto a 1.5% agar at 37°C for 15 minutes followed by addition of 0.5% agarose (Wako-Junyaku Co.) LB plate (containing 50  $\mu$  g/ml of

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then heated at 80 °C for 3 hours to fix DNAs.

Gene, Japan), 0.1% SDS and 100 $\mu$  g/ml of salmon sperm DNA for  $_{2}^{NaH_{2}PO_{4}\cdot H_{2}O}$ , 25 mM EDTA), 5 X Denhardt's solution (Nippon with the probe mentioned herein below in a buffer containing hybridization. 50% formamide, 5 x SSPE (20 x SSPE (pH 7.4) is 3 m NaC1, 0.2 M The filter was incubated overnight at 42 °C together

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10 Example 3, with EcoRI, followed by recovery and labelling by fragment inserted in the plasmid, p19P2, obtained in Working DNA labelling kit (Amasham Co.). incorporation of  $(^{32}{
m P}){
m dCTP}$  (Dupont Co.) with a random prime The probe used was obtained by cutting the DNA

5 then, subjected to an autoradiography at -80 °C to detect 0.3 M sodium citrate), 0.1% SDS at 55 °C for 1 hour and, hybridized plaques. It was washed with 2  $\times$  SSC (20  $\times$  SSC is 3 M NaCl,

25 30 prepared from the three clones. The DNAs digested with EcoRI 0.7kb, 0.8 kb and 2.0kb, respectively. Among them, the DNA by the southern blotting using the same probe as the one used were subjected to an agarose electrophoresis and were analyzed recognized in three independent plaques. Each DNA was hybridizable size was subcloned to the EcoRI site of the was selected. The  $\lambda$  hGR3-derived EcoRI fragment with a fragment corresponding to the band at about 2.0kb ( $\lambda$  hGR3) in the screening. Hybridizing bands were identified at about relying upon a restriction enzyme map deduced from the A restriction enzyme map of the plasmid, phGR3, was prepared plasmid, pUC18, and  $E_{\star}$  coli JM109 was transformed with the plasmid to obtain transformant E. coli JM109/phGR3. receptor protein-encoding DNA which was predicted from the As a result, it was learned that it carried a full-length nucleotide sequence as shown in Example 3. receptor protein-encoding DNA as shown in Example 3. In this screening, hybridization signals were

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 Sequencing of Human Pituitary Gland-Derived Receptor Protein CDNA. Among the EcoRI fragments inserted in the plasmid, phGR3, obtained in the above step (1), the from EcoRI to NheInucleotide sequence with about 1330bp that is considered to be a receptor protein-coding region was sequenced. Concretely speaking, by utilizing restriction enzyme sites that exist in the EcoRI fragments, unnecessary parts were removed or necessary fragments were subcloned in order to prepare template plasmids for analyzing the nucleotide sequence.

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The reaction for determining the nucleotide sequence (sequencing) was carried out with a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNA was decoded with the fluorescent automatic sequencer (ABI Co.), and the data of the nucleotide sequence obtained were analyzed with DNASIS (Hitachi System Engineering Co., Japan).

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Figure 34 shows a nucleotide sequence of from immediate after the EcoRI site up to the NheI site encoded by PhGR3. The nucleotide sequence of the human pituitary gland-derived receptor protein-encoding DNA corresponds to the nucleotide sequence of from 118th to 123rd nucleotides [Figure 34]. An amino acid sequence of the receptor protein that is encoded by the nucleotide sequence is shown in Figure 34. Figure 36 shows the results of hydrophobicity plotting based upon the amino acid sequence.

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(3) Northern Hybridization with Human Pituitary Gland-Derived Receptor Protein-Encoding phGR3

Northern blotting was carried out in order to detect the expression of phGR3-encoded human pituitary gland-derived receptor proteins in the pituitary gland at a mRNA level. Human pituitary gland mRNA (2.5  $\mu$  g, Clontech Co.) was used as a template mRNA and the same as the probe used in Working Example 5 was used as a probe. Nylon membrane (Pall Biodyne, U.S.A.) was used as a filter for northern blotting and migration of the mRNA and adsorption (sucking) thereof with the blotting filter was carried out according to the method as

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disclosed in Molecular Cloning, Cold Spring Harbor Laboratory Press, 1989. The hybridization was effected by incubating the above-mentioned filter and probe in a buffer containing 50% formamide, 5 x SSPE, 5 X Denhardt's solution, 0.1% SDS and 100 u g/ml of salmon sperm DNA overnight at 42 °C. The filter was washed with 0.1 x SSC, 0.1% SDS at 50 °C and, after drying with an air, was exposed to an X-ray film (XAR5, Kodak) for three days at -80 °C. The results were as shown in Figure 31 from which it is considered that the receptor gene encoded by phGR3 is expressed in the human pituitary gland.

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Example 7

Cloning of Mouse Pancreatic  $\beta$  -Cell Strain, MIN6-Derived G Protein Coupled Receptor Protein cDNA

(1) Preparation of Poly(A) \*RNA Fraction from Mouse Pancreatic  $\beta$  -Cell Strain, MIN6 and Synthesis of cDNA

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A total RNA was prepared from the mouse pancreatic \$\beta\$ -cell strain, MIN6 (Jun-ichi Miyazaki et al., Endocrinology, Vol. 127, No. 1, p.126-132) according to the guanidine thiocyanate method (Kaplan B.B. et al., Biochem. J., 183, 181-184 (1979)) and, then, poly(A)\*RNA fractions were prepared with a mRNA purifying kit (Pharmacia Co.). Next, to 5 \( \mu\) of the poly(A)\*RNA fraction was added a random DNA hexamer (BRL Co.) as a primer, and the resulting mixture was subject

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to reaction with MMLV reverse transcriptuse (BRL Co.) in the buffer attached to the MMLV reverse transcriptuse (BRL Co.) in the buffer attached to the MMLV reverse transcriptuse kit to synthesize complementary DNAs. The reaction product was extracted with phenol/chloroform (1:1), precipitated in ethanol, and was then dissolved in 30 µ 1 of TE.

(2) Amplification of Receptor cDNA by PCR Using MIN6-Derived CDNA and Sequencing

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By using, as a template, 5  $\mu$  1 of cDNA prepared from the mouse pancreatic  $\beta$  -cell strain, MIN6 in the above step (1), PCR amplification using the DNA primers synthesized in Example 1 was carried out. A reaction solution was

amplification including 96 °C for 30 sec., 45 °C for 1 min. polymerase, the remaining reaction solution was mixed and was and 60 °C for 3 min. was repeated 30 times by using a Thermal reaction solution was made to be 100  $\mu$  l. The cycle for 0.25 mM dNTPs, 1  $\mu$  l of Tag DNA polymerase and 10  $\mu$  l of 10× agarose gel electrophoresis and ethidium bromide staining. heated at 95 °C for 5 minutes and at 65 °C for 5 minutes. Cycler (Perkin-Elmer Co.). Prior to adding Tag DNA buffer attached to the enzyme kit, and the total amount of the and 3' primer sequence) each in an amount of 100 pm. The amplified products were confirmed relying upon 1.28 composed of the synthetic DNA primers (SEQ: 5' primer sequence

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Nucleotide Sequence of Inserted cDNA Region Selection of Novel Receptor Candidate Clone via Decoding Subcloning of PCR Product into Plasmid Vector and

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transformant Escherichia coli JM109/p3H2-17. E. coli JM109 competent cells (Takara Shuzo Co., Japan) to were separated by using a 0.8% low-melting temperature agarose white color were picked with a sterilized toothstick to obtain D-galactoside) and X-gal. Only transformant clones exhibiting culture medium containing ampicillin, IPTG (isopropylthio- $\beta$  a cDNA-inserted fragment were selected in an LB agar produce transformants. Then, transformant clones having the recovered DNAs were subcloned to the plasmid vector, protocol attached to a TA Cloning Kit (Invitrogen Co.), precipitated in ethanol to recover DNAs. According to the blade, and were heat-melted, extracted with phenol and gel, the band parts were excised from the gel with a razor II. The recombinant vectors were introduced into The PCR products obtained in the above step (2)

that was inserted. An aliquot of the remaining DNAs was was cut by EcoRI to confirm the size of the cDNA fragment prepare plasmid DNAs. An aliquot of the DNAs thus prepared automatic plasmid extracting machine (Kurabo Co., Japan) to LB culture medium containing ampicillin and treated with an The individual clones were cultured overnight in an

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fluorescent automatic sequencer, and the data of the sequencing kit (ABI Co.), the DNAs were decoded by using a was carried out by using a DyeDeoxy terminator cycle and precipitated in ethanol so as to be condensed. Sequencing further processed with RNase, extracted with phenol/chloroform

S nucleotide sequences obtained were read by using DNASIS (Hitachi System Engineering Co., Japan).

15 10 20 and at the amino acid sequence level to find homology relative was carried out in view of hydrophobicity plotting [Figure 38] Engineering Co., Japan) the nucleotide sequence were converted To further confirm this fact, by using DNASIS (Hitachi System was encoded by the cDNA fragment insert in the plasmid was learned that a novel G protein coupled receptor protein determined nucleotide sequence [Figure 37]. As a result, it (JN0605) and bovine neuropeptide Y receptor (S28787) to chicken ATP receptor (P34996), human somatostatin receptor into an amino acid sequence [Figure 37], and homology retrieval possessed by the transformant Escherichia coli JM109/p3H2-17. NBRF-PIR/Swiss-PROT and are usually called "Accession Numbers" numbers assigned when they are registered as data to [Figure 39]. Abbreviations in parentheses are reference subtype 3 (A46226), human somatostatin receptor subtype 4 Homology retrieval was carried out based upon the

### Example 8

Cloning of Mouse Pancreatic  $\beta$  -Cell Strain, MIN6-Derived G

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Protein Coupled Receptor Protein cDNA Preparation of Poly(A) RNA Fraction from Mouse Pancreatic eta -Cell Strain, MIN6 and Synthesis of cDNA

Vol. 127, No. 1, p.126-132) according to the guanidine  $extcolor{black}{eta}$  -cell strain, MIN6 (Jun-ichi Miyazaki et al., Endocrinology, A total RNA was prepared from the mouse pancreatic

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**υ** of the poly(A) RNA fraction was added a random DNA hexamer 181-184 (1979)) and, then, poly(A) RNA fractions were prepared thiocyanate method (Kaplan B.B. et al., Biochem. J., 183, with a mRNA purifying kit (Pharmacia Co.). Next, to 5  $\mu$  g (BRL Co.) as a primer, and the resulting mixture was subjected

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in the buffer attached to the MMLV reverse transcriptase kit was extracted with phenol/chloroform (1:1), precipitated in to synthesize complementary DNAs. The reaction product to reaction with MMLV reverse transcriptase (BRL Co.) ethanol, and was then dissolved in 30  $\mu$  1 of TE.

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Amplification of Receptor cDNA by PCR Using MIN6-Derived cDNA and Seguencing 2

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composed of the synthetic DNA primers (SEQ: 5' primer seguence buffer attached to the enzyme kit, and the total amount of the step (1), PCR amplification using the DNA primers synthesized and 60 °C for 3 min. was repeated 30 times by using a Thermal from the mouse pancreatic eta -cell strain, MIN6, in the above 0.25 mM dNTPs, 1  $\mu$  1 of Tag DNA polymerase and 10  $\mu$  1 of 10× amplification including 96 °C for 30 sec., 45 °C for 1 min. polymerase, the remaining reaction solution was mixed and agarose gel electrophoresis and ethidium bromide staining By using, as a template, 5  $\mu$  l of cDNA prepared heated at 95 °C for 5 minutes and at 65 °C for 5 minutes. The amplified products were confirmed relying upon 1.2% reaction solution was made to be  $100\,\mu$  l. The cycle for in Example 1 was carried out. A reaction solution was and 3' primer seguence) each in an amount of 100 pM, Cycler (Perkin-Elmer Co.). Prior to adding Tag DNA

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Selection of Novel Receptor Candidate Clone via Decoding Subcloning of PCR Product into Plasmid Vector and Nucleotide Sequence of Inserted cDNA Region 3

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were separated with a 0.8% low-melting temperature agarose gel, the band parts were excised from the gel with a razor precipitated in ethanol to recover DNAs. According to the The PCR products obtained in the above step (2) the recovered DNAs were subcloned to the plasmid vector, protocol attached to a TA Cloning Kit (Invitrogen Co.), E. coli JM109 competent cells (Takara Shuzo Co., Japan) blade, and were heat-melted, extracted with phenol and TM pcR II. The recombinant vectors were introduced into

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transformant clones exhibiting white color were picked with culture medium containing ampicillin, IPTG and X-gal. Only having a cDNA-inserted fragment were selected in an LB agar a sterilized toothstick to obtain transformant Escherichia to produce transformants. Then, transformant clones coli JM109/p3H2-34.

further processed with RNase, extracted with phenol/chloroform, and precipitated in ethanol so as to be condensed. Seguencing The individual clones were cultured overnight in an LB culture medium containing ampicillin and treated with an automatic plasmid extracting machine (Kurabo Co., Japan) to prepare plasmid DNAs. An aliquot of the DNAs thus prepared sequencing kit (ABI Co.), the DNAs were decoded by using a was cut by EcoRI to confirm the size of the cDNA fragment that was inserted. An aliquot of the remaining DNAs was nucleotide sequences obtained were read by using DNASIS was carried out by using a DyeDeoxy terminator cycle fluorescent automatic sequencer, and the data of the (Hitachi System Engineering Co., Japan).

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find homology relative to human somatostatin receptor subtype 2 NBRF-PIR/Swiss-PROT and are usually called "Accession Numbers" (Hitachi System Engineering Co., Japan) the nucleotide seguen JM109/p3H2-34. To further confirm this fact, by using DNASI3 plotting [Figure 41] and at the amino acid seguence level to determined nucleotide sequence [Figure 40]. As a result, it homology retrieval was carried out in view of hydrophobicity were converted into an amino acid sequence [Figure 40], and was learned that a novel G protein coupled receptor protein Homology retrieval was carried out based upon the (B41795) and rat-derived ligand unknown receptor (A39297) [Figure 42]. Abbreviations in parentheses are reference was encoded by the cDNA fragment insert in the plasmid numbers assigned when they are registered as data to possessed by the transformant Escherichia coli or "Entry Names".

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## Example 9

Cloning of Rabbit Gastropyrolic Part Smooth Muscle-Derived G Protein Coupled Receptor Protein cDNA

(1) Preparation of Poly(A) RNA Fraction from Rabbit

Gastropyrolic Part Smooth Muscle and Synthesis of cDNA

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A total RNA was prepared from rabbit gastropyrolic part smooth muscles by the guanidine thiocyanate method (Kaplan B.B. et al., Biochem. J. 183, 181-184 (1979)) and, then, poly(A) RNA fractions were prepared with a mRNA purifying kit (Pharmacia Co.). Next, to 5  $\mu$  g of the poly(A) RNA fraction was added a random DNA hexamer (BRL Co.) as a primer, and the resulting mixture was subjected to reaction with MMLV reverse transcriptase (BRL Co.) in the buffer attached to the MMLV reverse transcriptase kit to synthesize complementary DNAs. The reaction product was extracted with phenol/chloroform (1:1), precipitated in ethanol, and was then dissolved in 30  $\mu$  1 of TE (Tris-EDTA solution).

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(2) Amplification of Receptor cDNA by PCR Using Rabbit Gastropyrolic Part Smooth Muscle-Derived cDNA and Sequencing

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By using, as a template, 1 µ 1 of cDNA prepared from the rabbit gastropyrolic part smooth muscle in the above step (1), PCR amplification using the DNA primers synthesized in Example 1 was carried out. A reaction solution was composed of the synthetic DNA primers (SEQ: 5' primer sequence and 3' primer sequence) each in an amount of 100 pM, 0.25 mM dNTPs, 1 µ 1 of Taq DNA polymerase and 10 µ 1 of buffer attached to the enzyme kit, and the total amount of the reaction solution was made to be 100µ 1. The cycle for amplification including 96 °C for 30 sec., 45 °C for 1 min. and 60 °C for 3 min. was repeated 25 times by using a Thermal Cycler (Perkin-Elmer Co.). The amplified products were confirmed relying upon 1.2% agarose gel electrophoresis and ethidium bromide staining.

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(3) Subcloning of PCR Product into Plasmid Vector and Selection of Novel Receptor Candidate Clone via Decoding Nucleotide Sequence of Inserted cDNA Region The PCR products obtained in the above step (2)

15 S pCR II. The recombinant vectors were introduced into the recovered DNAs were subcloned to the plasmid vector, protocol attached to a TA Cloning Kit (Invitrogen Co.), precipitated in ethanol to recover DNAs. According to the blade, and were heat-melted, extracted with phenol and gel, the band parts were excised from the gel with a razor were separated with a 1.0% low-melting temperature agarose culture medium containing ampicillin, IPTG and X-gal. Only a cDNA-inserted fragment were selected in an LB agar produce transformants. Then, transformant clones having E. coli JM109 competent cells (Takara Shuzo Co., Japan) to a sterilized toothstick to obtain transformant Escherichia transformant clones exhibiting white color were picked with coli JM109/pMD4.

The individual clones were cultured overnight in an LB culture medium containing ampicillin and treated with an automatic plasmid extracting machine (Kurabo Co., Japan) to prepare plasmid DNAs. An aliquot of the DNAs thus prepared was cut by EcoRI to confirm the size of the cDNA fragment that was inserted. An aliquot of the remaining DNAs was further processed with RNase, extracted with phenol/chloroform, and precipitated in ethanol so as to be condensed. Sequencing was carried out by using a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNAs were decoded by using a fluorescent automatic sequencer, and the data of the

fluorescent automatic sequencer, and the data of the nucleotide sequences obtained were read by using DNASIS (Hitachi System Engineering Co., Japan). The determined nucleotide sequence was as shown in Figure 43. It was learned from Figure 43 that the cloned cDNA fragment was amplified from both sides with only the synthetic DNA primer having a nucleotide sequence represented by SEQ ID NO: 1 as synthesized in Example 1.

Homology retrieval was carried out based upon the

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determined nucleotide sequence [Figure 43]. As a result, it was learned that a novel G protein coupled receptor protein was encoded by the cDNA fragment insert in the plasmid possessed by the transformant Escherichia coli JM109/pMD4. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the nucleotide sequence were converted into an amino acid sequence [Figure 43], and homology retrieval was carried out in view of hydrophobicity plotting [Figure 44] and at the amino acid sequence level to find homology relative to rat ligand-unknown receptor protein (A35639) [Figure 45]. Abbreviations in parentheses are reference numbers assigned when they are registered as data to NBRF-PIR/Swiss-PROT and are usually called "Accession Numbers".

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# Example 10

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Cloning of cDNA Comprising Whole Coding Regions for Receptor Protein from Mouse Pancreatic  $\beta$  -Cell Strain, MIN6-Derived cDNA Library

(1) Cloning of cDNA Comprising Whole Coding Regions for Receptor Protein from Mouse Pancreatic  $\beta$  -Cell Strain, MIN6-Derived cDNA Library

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Superscript TM Lambda System (BRL, Cat. 8256) distributed by BRL Co. and Glgapack II Gold (Stratagene, Cat. 200215) distributed by Stratagene Co. were used to construct MIN6-derived cDNA libraries. By using the above kits, a MIN6 cDNA library with 2.2 x 10<sup>6</sup> pfu (plaque forming units) was constructed from 10µ g of MIN6 poly(A)\*RNA. The cDNA library was mixed with E. coli x1090 treated with magnesium sulfate, and incubated at 37 °C for 15 minutes followed by addition of 0.5% agarose (Pharmacia Co.) LB. The E. coli was plated onto a 1.5% agar (Wako-Junyaku Co.) LB plate (containing 50 µ g/ml of ampicillin). A nitrocellulose filter was placed on the plate on which plaques were formed and the plaque was transferred onto the filter. The filter was denatured with an alkali and then heated at 80 °C for 3 hours to fix DNAs.

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The filter was incubated overnight at 42 °C together

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with the probe mentioned herein below in a buffer containing 50% formamide, 5 x SSPE, 5 X Denhardt's solution, 0.1% SDS and 100  $\mu$  g/ml of salmon sperm DNA for hybridization.

The probe used was obtained by cutting the DNA fragment inserted in the plasmid, p3H2-34, obtained in Working Example 8, with EcoRI, followed by recovery and labeling by incorporation of [\$^{32}\$]dCTP (Dupont Co.) with a random prime DNA labelling kit (Amasham Co.).

It was washed with 2 x SSC (150 mM NaCl and 15 mM sodium citrate), 0.1% SDS at 55 °C for 1 hour and, then, subjected to an autoradiography at -80 °C to detect hybridized plaques.

prepared from the two clones. The DNAs digested with Sall and full-length receptor protein-encoding DNA which was predicted map deduced from the nucleotide sequence as shown in Working plasmid, pMGR20, was prepared relying upon a restriction enz fragment corresponding to the band at about 3.0kb (1 No.20) NotI were subjected to an agarose electrophoresis and were was selected. The  $\lambda$  No.20-derived NotI-Sall fragment with from the receptor protein-encoding DNA as shown in Working Example 8. As a result, it was learned that it carried a about 3.0kb was subcloned into the NotI-SalI site of the In this screening, hybridization signals were analyzed. Inserted fragments were identified at about E. coli JM109/pMGR20. A restriction enzyme map of the 2.0kb and 3.0kb, respectively. Between them, the DNA plasmid, pBluescript  $^{TM}$  II SK(+), and  $\stackrel{E.}{\text{coli}}$  JM109 was ecognized in two independent plagues. Each DNA was transformed with the plasmid to obtain a transformant

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(2) Sequencing of MIN6-Derived Receptor Protein Full-Length CDNA Among the NotI-SalI fragments inserted in the plasmid, pMGR20, obtained in the above step (1), the nucleotide sequence with total 1607bp, including not only a region that is considered to be a receptor protein-coding region (ORF) but

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also a neighboring region thereof was sequenced. Concretely template plasmids for analyzing the nucleotide sequence thereof. necessary fragments were subcloned in order to prepare the NotI-SalI fragments, unnecessary parts were removed or speaking, by utilizing restriction enzyme sites that exist in sequences that were determined already and used to make for sequencing were synthesized based upon the nucleotide As for the nucleotide sequences of part of the regions, primers

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cycle sequencing kit (ABI Co.), the DNA was decoded with confirmation. (Hitachi System Engineering Co., Japan). the nucleotide sequence obtained were analyzed with DNASIS the fluorescent automatic sequencer (ABI Co.), and the data of (sequencing) was carried out with a DyeDeoxy terminator The reaction for determining the nucleotide sequence

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reading frame (ORF) of a mouse galanin receptor protein nucleotide sequence in Figure 46. The nucleotide sequence was corresponds to from the 481st to 1525th nucleotides of the sequence of mouse galanin receptor protein-encoding DNA encoded by the cDNA insert in pMGR20. The nucleotide protein-encoding cDNA. insert in the pMGR20 is a mouse-derived galanin receptor sequence level [Figure 48], it was learned that the cDNA human-derived galanin receptor protein at the amino acid the amino acid sequence [Figure 46] has 92% homology to the hydrophobicity plotting was carried out [Figure 47]. Since converted into an amino acid sequence [Figure 46] and Figure 46 shows a nucleotide sequence around an open

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Preparation of Synthetic DNA Primer for Amplifying G Protein Coupled Receptor Protein-Encoding DNA Highly homologous parts were found by comparing

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G protein coupled receptors, i.e., rat-derived angiotensin II membrane-spanning domain [6C of Figure 6] among known membrane-spanning domain [3C and 3D in Figure 4] and the sixth nucleotide seguences corresponding to or near the third

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protein (X64052), rat-derived angiotensin receptor protein subtype (M90065), human-derived angiotensin Ia receptor protein (M88096), rat-derived cholecystokinin $_{
m B}$  receptor protein (M91464), rat-derived cholecystokinin $_{
m A}$  receptor protein receptor protein (L32840), rat-derived angiotensin Ib receptor

protein (M73969), human-derived high-affinity interleukin 8 (M99418), human-derived cholecystokinin $_{
m B}$  receptor protein receptor protein (M60626), etc. receptor protein (S46665), human-derived N-formyl peptide receptor protein (x65858), mouse-derived C5a anaphylatoxin (L04473), mouse-derived low-affinity interleukin 8 receptor

data base is retrieved, and are usually called "Accession reference numbers that are indicated when the GenBank/EMBL The aforementioned abbreviations in parentheses are

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25 even in other regions. Based upon these seguences, the upon the base regions that were in agreement with a large degenerate synthetic DNA (3D of Figure 4) having a nucleotide agreement of sequences with as many receptor cDNAs as possible number of receptor protein cDNAs in order to enhance base sequence represented by SEQ ID NO: 3 which is complementary to SEQ ID NO: 4 were produced. Nucleotide synthesis was carried 6C of rigure 6) having a nucleotide sequence represented by degenerate synthetic DNA (nucleotide sequence complementary to the homologous nucleotide sequence of Figure 4 and the out by a DNA synthesizer. It was planned to incorporate mixed bases relying

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[Synthetic DNAs]

5'-CTCGC (G or C) GC (C or T) (A or C) TI (A or G) G

ä (C or T) ATGGA (C or T) CGITAT-3'

5'-CATGT (A or G) G (T or A) AGGGAAICCAG (G or C) A (A or C) AI (A or G) A (A or G)(A or G) AA-3'

(SEQ ID NO:4)

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plurality of bases, leading to multiple oligonucleotides in the parentheses of the aforementioned DNAs were incorporated in the presence of a mixture of plural bases at the time of synthesis, primer preparation. In other words, nucleotide residues in The parentheses indicate the incorporation of a provided that I denotes inosine.

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# Example 12

Cloning of Rabbit Gastropyrolic Part Smooth Muscle-Derived G Protein Coupled Receptor Protein CDNA

Gastropyrolic Part Smooth Muscle and Synthesis of cDNA (1) Preparation of Poly(A) \*RNA Fraction from Rabbit

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(BRL Co.) as a primer, and the resulting mixture was subjected A total RNA was prepared from rabbit gastropyrolic in the buffer attached to the MMLV reverse transcriptase kit was extracted with phenol/chloroform (1:1), precipitated in (Kaplan B.B. et al., Biochem. J. 183, 181-184 (1979)) and, part smooth muscles by the guanidine thiocyanate method to synthesize complementary DNAs. The reaction product then, poly(A)  $^{\star}$ RNA fractions were prepared with a mRNA purifying kit (Pharmacia Co.). Next, to 5  $\mu$  g of the to reaction with MMLV reverse transcriptase (BRL Co.) poly(A) \*RNA fraction was added a random DNA hexamer ethanol, and was then dissolved in 30  $\mu$  1 of TE.

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(2) Amplification of Receptor cDNA by PCR Using Rabbit Gastropyrolic Part Smooth Muscle-Derived cDNA and Sequencing

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from the rabbit gastropyrolic part smooth muscle in the above A reaction solution was composed of the synthetic DNA primers amount of 100 pM, 0.25 mM dNTPs, 1  $\mu$  l of Tag DNA polymerase (SEQ: 5' primer sequence and 3' primer sequence) each in an nucleotide sequence represented by SEQ ID NO: 3 and the DNA step (1), PCR amplification using the DNA primer having a By using, as a template, 1  $\mu$  1 of cDNA prepared primer having a nucleotide sequence represented by SEQ ID NO: 4 synthesized in Example 11 was carried out.

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The cycle for amplification including 96  $^{\circ}$ C for 30 sec., 45  $^{\circ}$ C for 1 min. and 60  $^{\circ}$ C for 3 min. was repeated 25 times by using total amount of the reaction solution was made to be 100  $\mu$  1. a Thermal Cycler (Perkin-Elmer Co.). The amplified products were confirmed relying upon 1.2% agarose gel electrophoresis and 10  $\mu$  1 of buffer attached to the enzyme kit, and the and ethidium bromide staining.

Selection of Novel Receptor Candidate Clone via Decoding Subcloning of PCR Product into Plasmid Vector and Nucleotide Seguence of Inserted cDNA Region 3

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transformant clones exhibiting white color were picked with culture medium containing ampicillin, IPTG and X-gal. Only were separated with a 1.0% low-melting temperature agarose gel, the band parts were excised from the gel with a razor precipitated in ethanol to recover DNAs. According to the a sterilized toothstick to obtain transformant Escherichia E. coli JM109 competent cells (Takara Shuzo Co., Japan) to The PCR products obtained in the above step (2) produce transformants. Then, transformant clones having the recovered DNAs were subcloned to the plasmid vector, blade, and were heat-melted, extracted with phenol and Protocol attached to a TA Cloning Kit (Invitrogen Co.),  $^{
m TM}$  DCR II. The recombinant vectors were introduced into a cDNA-inserted fragment were selected in an LB agar coli JM109/pMJ10.

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further processed with RNase, extracted with phenol/chloroform, and precipitated in ethanol so as to be condensed. Sequencing The individual clones were cultured overnight in an LB culture medium containing ampicillin and treated with an automatic plasmid extracting machine (Kurabo Co., Japan) to prepare plasmid DNAs. An aliquot of the DNAs thus prepared sequencing kit (ABI Co.), the DNAs were decoded by using a was cut by EcoRI to confirm the size of the cDNA fragment that was inserted. An aliquot of the remaining DNAs was was carried out by using a DyeDeoxy terminator cycle fluorescent automatic sequencer, and the data of the

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nucleotide sequence was as shown in Figure 49. nucleotide sequences obtained were read by using DNASIS (Hitachi System Engineering Co., Japan). The determined

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15 20 determined nucleotide sequence [Figure 49]. As a result, JM109/pMJ10. To further confirm this fact, by using DNASIS plasmid possessed by the transformant Escherichia coli protein was encoded by the cDNA fragment insert in the it was learned that a novel G protein coupled receptor plotting [Figure 50] and at the amino acid sequence level to homology retrieval was carried out in view of hydrophobicity were converted into an amino acid sequence [Figure 49], and (Hitachi System Engineering Co., Japan) the nucleotide sequence Y receptor protein (S28787) [Figure 51]. Abbreviations in anaphylatoxin receptor protein (A46525) and bovine neuropeptide (B42009), human N-formyl peptide receptor protein (JC2014), find homology relative to human ligand unknown receptor protein called "Accession Numbers". registered as data to NBRF-PIR/Swiss-PROT and are usually parentheses are reference numbers assigned when they are rabbit N-formyl peptide receptor protein (A46520), mouse C5a Homology retrieval was carried out based upon the

Coupled Receptor Protein-Encoding DNA Preparation of Synthetic DNA Primer for Amplifying G Protein

 $\mu$  -opioid receptor protein (D16349), mouse-derived bradykinin derived  $\delta$  -opioid receptor protein (L11065), rat-derived mouse-derived  $\kappa$  -opioid receptor protein (L11064), mousedomain among known G protein coupled receptors, i.e., regions corresponding to or near the third membrane-spanning A comparison of nucleotide sequences coding for

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receptor protein (M599967), mouse-derived bombesin receptor B2 receptor protein (x69676), rat-derived bradykinin B2 subtype 3 (L08893), mouse-derived substance K receptor protein protein (M73481), human-derived bombesin receptor protein (M73482), human-derived gastrin releasing peptide receptor protein (M35328), human-derived neuromedin B receptor protein

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unknown receptor proteins (L04672), (X61496), (X59249) and endothelin receptor protein (M60786), rat-derived ligand rat-derived neurokinin 3 receptor protein (J05189), rat-derived (L09249), mouse-derived ligand unknown receptor protein (highly homologous nucleotides) was synthesized to enhance (3B in Figure 3; SEQ ID NO: 6) with highly common bases the degenerate DNA primer having a nucleotide sequence (M31210) and (U03642), etc. was made. In particular, (x62933), mouse-derived substance P receptor protein (x62934), sequence regions that were in agreement with a large number of possible even in other regions on the basis of nucleotide base agreement of sequences with as many receptor cDNAs as (P30731), human-derived ligand unknown receptor proteins receptor cDNAs. Nucleotide synthesis was carried out by a DNA

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The nucleotide sequence represented by SEQ ID NO: 6

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5'-CTGAC (C or T) G (C or T) TCTI (A or G)(G or C) I (A or G)(C or T) TGAC (A or C) G (A, C or G) TAT-3'

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primer preparation. In other words, nucleotide residues in provided that I denotes inosine. presence of a mixture of plural bases at the time of synthesis, parentheses of the aforementioned DNAs were incorporated in the plurality of bases, leading to multiple oligonucleotides in the The parentheses indicate the incorporation of a

bradykinin B2 receptor protein (X69676), rat-derived bradykinin (L11064), mouse-derived & -opioid receptor protein (L11065), receptors, i.e., mouse-derived & -opioid receptor protein membrane-spanning domain among known G protein coupled coding for regions corresponding to or near the sixth protein (M35328), human-derived neuromedin B receptor protein B2 receptor protein (M59967), mouse-derived bombesin receptor rat-derived u -opioid receptor protein (D16349), mouse-derived furthermore, a comparison of nucleotide sequences

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rat-derived neurokinin 3 receptor protein (J05189), rat-derived subtype 3 (L08893), mouse-derived substance K receptor protein (X62933), mouse-derived substance P receptor protein (X62934), agreement of sequences with as many receptor cDNAs as possible even in other portions on the basis of base regions that are (M73482), human-derived gastrin releasing peptide receptor unknown receptor proteins (L04672), (X61496), (X59249) and sequence (6A in Figure 5) with highly common bases (highly protein (M73481), human-derived bombesin receptor protein endothelin receptor protein (M60786), rat-derived ligand (P30731), human-derived ligand unknown receptor proteins (L09249), mouse-derived ligand unknown receptor protein homologous nucleotides) was synthesized to enhance base (SEQ ID NO: 8) which is complementary to the nucleotide the degenerate DNA primer having a nucleotide seguence (M31210) and (U03642), etc. was made. In particular, in agreement with a large number of receptor cDNAs.

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The nucleotide sequence represented by SEQ ID NO: 8

5'-GATGTG (A or G) TA (A or G) GG (G or C)(A or G)
ICCAACAGAIG (A or G) (C or T) AAA-3'

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The parentheses indicate the incorporation of a plurality of bases, leading to multiple oligonucleotides in the primer preparation. In other words, nucleotide residues in parentheses of the aforementioned DNAs were incorporated in the presence of a mixture of plural bases at the time of synthesis, provided that I denotes inosine.

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The aforementioned abbreviations in parentheses are reference numbers indicated when the GenBank/EMBL data base is retrieved and are usually called "Accession Numbers".

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### Example 14

Cloning of Rabbit Gastropyrolic Part Smooth Muscle-Derived G Protein Coupled Receptor Protein cDNA

 Preparation of Poly(A) \*RNA Fraction from Rabbit Gastropyrolic Part Smooth Muscle and Synthesis of cDNA

A total RNA was prepared from rabbit gastropyrolic part smooth muscles by the guanidine thiocyanate method (Kaplan B.B. et al., Biochem. J. 183, 181-184 (1979)) and, then, poly(A) RNA fractions were prepared with a mRNA purifying kit (Pharmacia Co.). Next, to 5 u g of the poly(A) RNA fraction was added a random DNA hexamer (BRL Co.) as a primer, and the resulting mixture was subjected to reaction with MMLV reverse transcriptase (BRL Co.) in the buffer attached to the MMLV reverse transcriptase kit to synthesize complementary DNAs. The reaction product was extracted with phenol/chloroform (1:1), precipitated in ethanol, and was then dissolved in 30 u 1 of TE.

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(2) Amplification of Receptor cDNA by PCR Using Rabbit Gastropyrolic Part Smooth Muscle-Derived cDNA and Sequencing

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By using, as a template, 1  $\mu$  1 of cDNA prepared from the rabbit gastropyrolic part smooth muscle in the above step (1), PCR amplification using the DNA primer having a nucleotide sequence represented by SEQ ID NO: 6 and the DNA primer having a nucleotide sequence represented by SEQ ID NO: 8 synthesized in Example 13 was carried out.

A reaction solution was composed of the synthetic DNA primers (SEQ: 5' primer sequence and 3' primer sequence) each in an amount of 100 pM, 0.25 mM dNTPs, 1  $\mu$  1 of Tag DNA polymerase and 10  $\mu$  1 of buffer attached to the enzyme kit, and the total amount of the reaction solution was made to be  $100\mu$  1. The cycle for amplification including 96 °C for 30 sec., 45 °C for 1 min. and 60 °C for 3 min. was repeated 25 times by using a Thermal Cycler (Perkin-Elmer Co.). The amplified products were confirmed relying upon 1.2% agarose gel electrophoresis

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and ethidium bromide staining.

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Nucleotide Sequence of Inserted cDNA Region Selection of Novel Receptor Candidate Clone via Decoding Subcloning of PCR Product into Plasmid Vector and

a sterilized toothstick to obtain transformant Escherichia transformant clones exhibiting white color were picked with culture medium containing ampicillin, IPTG and X-gal. Only a cDNA-inserted fragment were selected in an LB agar produce transformants. Then, transformant clones having E. coli JM109 competent cells (Takara Shuzo Co., Japan) to  $\mathsf{pCR}^{\mathsf{TM}}$  II. The recombinant vectors were introduced into the recovered DNAs were subcloned to the plasmid vector, coli JM109/pMH28. protocol attached to a TA Cloning Kit (Invitrogen Co.), precipitated in ethanol to recover DNAs. According to the blade, and were heat-melted, extracted with phenol and gel, the band parts were excised from the gel with a razor were separated by using a 1.0% low-melting temperature agarose The PCR products obtained in the above step (2)

nucleotide sequences obtained were read by using DNASIS and precipitated in ethanol so as to be condensed. Sequencing nucleotide sequence was as shown in Figure 52. fluorescent automatic sequencer, and the data of the was carried out by using a DyeDeoxy terminator cycle further processed with RNase, extracted with phenol/chloroform, that was inserted. An aliquot of the remaining DNAs was LB culture medium containing ampicillin and treated with an (Hitachi System Engineering Co., Japan). The determined sequencing kit (ABI Co.), the DNAs were decoded by using a was cut by EcoRI to confirm the size of the cDNA fragment prepare plasmid DNAs. An aliquot of the DNAs thus prepared automatic plasmid extracting machine (Kurabo Co., Japan) to The individual clones were cultured overnight in an

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plasmid possessed by the transformant Escherichia coli protein was encoded by the cDNA fragment insert in the it was learned that a novel G protein coupled receptor determined nucleotide seguence [Figure 52]. As a result, Homology retrieval was carried out based upon the

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or SWISS-PROT and are usually called "Accession Numbers". numbers assigned when they are registered as data to NBRF-PIR The aforementioned abbreviations in parentheses are reference somatostatin receptor protein 4 (A47457)[Figure 54]. human somatostatin receptor protein 1 (A41795) and human find homology relative to mouse IL-8 receptor protein (P35343), plotting [Figure 53] and at the amino acid sequence level to homology retrieval was carried out in view of hydrophobicity were converted into an amino acid sequence [Figure 52], and JM109/pMH28. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the nucleotide sequence

Coupled Receptor Protein-Encoding DNA Preparation of Synthetic DNA Primer for Amplifying G Protein

15 eta -1-adrenergic receptor (HUMADRB1), rabbit-derived IL-8 lpha -1B-adrenergic receptor (RATADR1B), human-derived human-derived galanin receptor (HUMGALAREC), rat-derived (HUMOPIODRE), bovine-derived substance K receptor (BTSKR), receptor (RABIL8RSB), human-derived opioid receptor domain among known G protein coupled receptors, i.e., regions corresponding to or near the second membrane-spanning A comparison of nucleotide sequences coding for

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8 25 mouse-derived serotonin receptor-2 (MMSERO), rat-derived (HUM5HT1E), human-derived dopamine receptor D4 (HUMD4C), receptor-D5 (HUMDIB), human-derived serotonin receptor 5HT1E cholecystokinin A receptor (HUMCCKAR), human-derived dopamine human-derived gastrin receptor (HUMGARE), human-derived human-derived somatostatin receptor-3 (HUMSSTR3Y),

human-derived somatostatin receptor-2 (HUMSTRI2A),

even in other regions on the basis of nucleotide sequence agreement of sequences with as many receptor cDNAs as possible homologous nucleotides) was synthesized to enhance base Figure 7, SEQ ID NO: 10) with highly common bases (highly H2 receptor (S57565), etc. was made. In particular, the a -lA-adrenergic receptor (RATADRAlA), rat-derived histamine degenerate DNA primer having a nucleotide sequence (T2A in

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regions that were in agreement with a large number of receptor cDNAs. Nucleotide synthesis was carried out by a DNA synthesizer.

The nucleotide sequence represented by SEQ ID NO: 10

is:

5'-GYCACCAACN WSTTCATCCTSWN HCTG-3'

wherein S represents G or C; Y represents C or T; W represents A or T; H represents A, C or T and N, represents I.

The parentheses indicate the incorporation of a plurality of bases, leading to multiple oligonuclectides in the primer preparation. In other words, nucleotide residues in parentheses of the aforementioned DNAs were incorporated in the presence of a mixture of plural bases at the time of synthesis, provided that I denotes inosine.

mouse-derived somatostatin receptor 1 (MUSSRI1A), human-derived unknown receptor (859748), human-derived somatostatin receptor rat-derived Al adenosine receptor (RATIADREC), porcine-derived angiotensin receptor (PIGA2R), rat-derived serotonin receptor receptors, i.e., human-derived galanin receptor (HUMGALAREC), (HUMSST28A), rat-derived ligand unknown receptor (RNGPROCR), mouse-derived GRP/bombesin receptor (MUSGRPBOM), rat-derived Furthermore, a comparison of nucleotide sequences human-derived gastrin releasing peptide receptor (HUMGRPR), human-derived muscarinic acetylcholine receptor (HSHM4), cholecystokinin receptor (RATCCKAR), rat-derived ligand coding for regions corresponding to or near the seventh membrane-spanning dòmain among known G protein coupled (RATSHTRTC), human-derived dopamine receptor (S58541), human-derived gastrin receptor (HUMGARE), rat-derived a -Al-adrenergic receptor (HUMALAADR), mouse-derived human-derived eta -1 adrenergic receptor (HUMDRB1), vascular type 1 angiotensin receptor (RRVTlAIIR),

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receptor-3 (HUMSSTR3Y), etc. was made. In particular, the degenerate DNA primer having a nucleotide sequence (T7A in Figure 8, SEQ ID NO: 11) with highly common bases (highly homologous nucleotides) was synthesized to enhance base agreement of sequences with as many receptor cDNAs as possible even in other regions on the basis of nucleotide sequence regions that were in agreement with a large number of receptor cDNAs. Nucleotide synthesis was carried out by a DNA synthesizer.

The nucleotide sequence represented by SEQ ID NO: 11 is: 5'-asn,san,raagsartagan<sub>2</sub>Gan<sub>2</sub>RGGRTT-3'

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wherein R represents A or G; S represents G or C and  $\rm N_2$  represents I.

The parentheses indicate the incorporation of a plurality of bases, leading to multiple oligonucleotides in the primer preparation. In other words, nucleotide residues in parentheses of the aforementioned DNAs were incorporated in the presence of a mixture of plural bases at the time of synthesis, provided that I denotes inosine.

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The aforementioned abbreviations in parentheses are reference numbers indicated when the GenBank/EMBL data base retrieved and are usually called "Accession Numbers".

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### xample 16

- 25 Cloning of Rabbit Gastropyrolic Part Smooth Muscle-Derived G Protein Coupled Receptor Protein CDNA
  - (1) Preparation of Poly(A) RNA Fraction from Rabbit
    Gastropyrolic Part Smooth Muscle and Synthesis of CDNA
    A total RNA was prepared from rabbit gastropyrolic
    - 30 part smooth muscles by the guanidine thiocyanate method (Kaplan B.B. et al., Biochem. J. 183, 181-184 (1979)) and, then, poly(A) RNA fractions were prepared with a mRNA purifying kit (Pharmacia Co.). Next, to 5  $\mu$  9 of the

delta-opioid receptor (S66181), human-derived somatostatin

poly(A) RNA fraction was added a random DNA hexamer (BRL Co.) as a primer, and the resulting mixture was subjected to reaction with MMLV reverse transcriptase (BRL Co.) in the buffer attached to the MMLV reverse transcriptase kit to synthesize complementary DNAs. The reaction product was extracted with phenol/chloroform (1:1), precipitated in ethanol, and was then dissolved in 30  $\mu$  l of TE.

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(2) Amplification of Receptor cDNA by PCR Using Rabbit Gastropyrolic Part Smooth Muscle-Derived cDNA and Sequencing

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confirmed relying upon 1.2% agarose gel electrophoresis and A reaction solution was composed of the synthetic DNA primers ethidium bromide staining. Thermal Cycler (Perkin-Elmer Co.). The amplified products were The cycle for amplification including 96 °C for 30 sec., 45 °C total amount of the reaction solution was made to be 100  $\mu$  1. and 10  $\mu$  l of buffer attached to the enzyme kit, and the NO: 11 synthesized in Example 15 was carried out. primer having a nucleotide sequence represented by SEQ ID nucleotide sequence represented by SEQ ID NO: 10 and the DNA step (1), PCR amplification using the DNA primer having a for 1 min. and 60 °C for 3 min. was repeated 25 times with a amount of 100 pM, 0.25 mM dNTPs, 1  $\mu$  l of Tag DNA polymerase (SEQ: 5' primer sequence and 3' primer sequence) each in an from the rabbit gastropyrolic part smooth muscle in the above By using, as a template, 1  $\mu$  l of cDNA prepared

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(3) Subcloning of PCR Product into Plasmid Vector and Selection of Novel Receptor Candidate Clone via Decoding Nucleotide Seguence of Inserted cDNA Region

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The PCR products obtained in the above step (2) were separated with a 1.4% low-melting temperature agarose gel. the band parts were excised from the gel with a razor blade, and were eluted electrophoretically, extracted with phenol and precipitated in ethanol to recover DNAs. According to the protocol attached to a TA Cloning Kit (Invitrogen Co.),

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the recovered DNAs were subcloned to the plasmid vector, pCR II. The recombinant vectors were introduced into E. coli JM109 competent cells (Takara Shuzo Co., Japan) to produce transformants. Then, transformant clones having a cDNA-inserted fragment were selected in an LB agar culture medium containing ampicillin, IPTG and X-gal. Only transformant clones exhibiting white color were picked with a sterilized toothstick to obtain 100 transformant clones.

The individual clones were cultured overnight in an LB culture medium containing ampicillin and treated with the automatic plasmid extracting machine PI-100 (Kurabo Co., Japan) to prepare plasmid DNAs. An aliquot of the DNA thus prepared was cut by ECORI to confirm the size of the cDNA fragment that was inserted. An aliquot of the remaining DNA was further processed with RNase, extracted with phenol/chloroform, and precipitated in ethanol so as to be condensed. Sequencing was carried out by using a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNAs were decoded by using a fluorescent automatic sequencer.

ω S 30 25 20 to rat-derived serotonin (5-HT6) receptor protein (JN0591), proteins having 27% homology relative to rat-derived acid sequence level to find that the DNAs were novel receptor As a result, the presence of hydrophobic domains which prove and hydrophobicity plotting was carried out [Figure 57]. converted into amino acid sequences [Figure 55] and [Figure 56], To further confirm this fact, by using DNASIS (Hitachi System the transformant Escherichia coli JM109/pMN7. Figure 56 and  $eta_3$ -adrenaline receptor protein (A41679), 29% homology relative Furthermore, homology retrieval was carried out at the amino that it is a G protein coupled receptor protein were confirmed. Engineering Co., Japan), the nucleotide seguences were Figure 56 show the nucleotide sequences of the cDNA fragments. novel G protein coupled receptor protein was encoded by Engineering Co., Japan). As a result, it was learned that a the cDNA fragment insert in the plasmid possessed by determined nucleotide sequence by using DNASIS (Hitachi System Homology retrieval was carried out based upon the

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27% homology relative to dog-derived histamine H<sub>2</sub> receptor protein (A39008), 27% homology relative to human-derived somatostatin receptor (type 4) protein (JN0605), 24% homology relative to human-derived dopamine D<sub>1</sub> receptor protein (S11377), 23% homology relative to rat-derived neurotensin receptor protein (JH0164), 31% homology relative to human-derived cholecystokinin B receptor protein (JC1352), and 30% homology relative to rat-derived gastrin receptor protein (JQ1614). The aforementioned abbreviations in protein (JQ1614). The aforementioned abbreviations in parentheses are reference numbers assigned when they are registered as data to NBRF-PIR and are usually called "Accession Numbers".

### Example 17

Amplification of Receptor CDNA by PCR Using MIN6-Derived CDNA and Sequencing

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By using, as a template, 5  $\mu$  1 of cDNA prepared from the mouse pancreatic  $\beta$  -cell strain, MIN6 in Working Example 4 (1), PCR amplification using the DNA primers synthesized in Example 4 (2) as disclosed in Libert F. et al., "Science, 244:569-572, 1989", i.e., a synthetic primer represented by the following sequence:

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5'-CTGTG (C or T) G (C or T) (G or C) AT (C or T) GCIIT
(G or T) GA (C or T) (A or C) G (G or C) TAC-3'
(SEQ ID NO: 60)

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25 wherein I is inosine; and a synthetic primer represented by the following sequence: 5'-A (G or T) G (A or T) AG (A or T) AGGCCAGCAGAI
(G or C) (A or G) (C or T) GAA-3'
(SEQ ID NO: 61)
wherein I is inosine, was carried out under the same conditions

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wherein I is inosine, was carried out under the same condition as in Example 3 (1). The resulting PCR product was subcloned to the plasmid vector, pCR  $^{\mathrm{TM}}$ II, in the same manner as in Example 3 (2) to obtain a plasmid, p5S38. The plasmid p5S38 was transfected into  $^{\mathrm{E}}$ .  $^{\mathrm{coli}}$  JM109 to obtain

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transformant Escherichia coli JM109/p5538.

The reaction for determining the nucleotide sequence (sequencing) was carried out with a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNA was decoded with the fluorescent automatic sequencer (ABI Co.), and the data of the nucleotide sequence obtained were read with DNASIS (Hitachi System Engineering Co., Japan).

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Figure 62 shows a mouse pancreatic  $\beta$  -cell strain MIN6-derived G protein coupled receptor protein-encoding DNA (SEQ ID NO: 33) and an amino acid sequence (SEQ ID NO: 28) encoded by the isolated DNA based upon the nucleotide sequence of plasmid, p5S38. The underlined portions represent regions corresponding to the synthetic primers.

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Homology retrieval was carried out based upon the determined nucleotide sequence (Figure 62]. As a result, it was learned that a novel G protein coupled receptor protein was encoded by the cDNA fragment obtained. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan), the nucleotide sequence was converted into an amino acid sequence (Figure 62), and hydrophobicity plotting was carried out to confirm the presence of four hydrophobic regions (Figure 64). Upon comparing the amino acid sequence with those encoded by p19P2 obtained in Example 3 (2) and encoded by pG3-2 obtained in Example 4 (2), furthermore,

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a high degree of homology was found as shown in Figure 63. As a result, it is strongly suggested that the mouse pancreatic  $\beta$  -cell strain, MIN6-derived G protein coupled receptor protein encoded by p5538 recognizes the same ligand as the human pituitary gland-derived G protein coupled receptor protein encoded by p19P2 does while the animal species from which the receptor protein encoded by p5538 is derived is different from that from which the receptor protein encoded by p19P2 is. It is also strongly suggested that the mouse pancreatic  $\beta$  -cell strain, MIN6-derived G protein coupled receptor protein encoded by p5538 recognizes the same ligand as the mouse pancreatic  $\beta$  -cell strain, MIN6-derived G protein coupled receptor proteins encoded by p5538 recognizes the same ligand as the mouse pancreatic  $\beta$  -cell strain, MIN6-derived G protein coupled receptor proteins encoded by p63-2 and pG1-10 do and

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they are analogous receptor proteins one another (so-called

MIN6-Derived Receptor Protein-Encoding p3H2-17 Northern Hybridization with cDNA Fragment Included in

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(2.5 $\mu$  g) were used as poly(A) RNAs. The DNA fragment inserted and mouse brain, spleen, thymus and pancreas poly(A) RNAs Mouse cell line, MIN6, Neuro-2a, poly(A) RNA (2.5  $\mu$  g)

was recovered as a DNA fragment with about 400bp by cutting labeled by incorporation of  $[^{32}_{P}]dCTP$  (Dupont Co.) with a the plasmid with EcoRI and the resulting DNA fragment was into the plasmid, p3H2-17, obtained in Example 7 (3) random prime DNA labeling kit (Amasham Co.). The about 400bp labeled DNA fragment was used as a probe for hybridization.

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filter for northern blotting and migration of the poly(A) RNA Cloning, Cold Spring Harbor Laboratory Press, 1989. carried out according to the method as disclosed in Molecular and adsorption (sucking) thereof with the blotting filter was Nylon membrane (Pall Biodyne, U.S.A.) was used as a

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overnight at 42 °C. The filter was washed with 0.1 x SSC Gene, Japan), 0.1% SDS and  $100\,\mu$  g/ml of salmon sperm DNA  $_{\rm NaH_2PO_4^{+}H_2^{-}O}$ , 25 mM EDTA), 5 X Denhardt's solution (Nippon above-mentioned filter and probe in a buffer containing 50% 50 °C and, after drying with an air, was exposed to an X-ray formamide, 5 x SSPE (20 x SSPE (pH 7.4) is 3 M NaC1, 0.2 M film (XAR5, Kodak) for 15 days at -80 °C. The results were as (20 x SSC is 3 M NaCl, 0.3 M sodium citrate), 0.1% SDS at shown in Figure 65. The hybridization was carried out by incubating the

plotting is different from those of other organ cells. signal position hybridizable in the northern hybridization expressed in the mouse spleen and thymus intensely. The MIN6 the mouse brain, pancreas, spleen and thymus and especially р3H2-17 is expressed in the cell line, MIN6, Neuro-2a, and the receptor gene encoded by the cDNA fragment included in It is considered from Figure 65 that mRNA for the

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Example 19

PCR Cloning of cDNA Comprising Whole Coding Regions of Receptor Proteins from Mouse Spleen, Thymus-Derived

Poly(A) RNA and Sequencing

S PCR Cloning of cDNA Comprising Whole Coding Region of Receptor Protein

by 5'RACE and 3'RACE wherein poly(A) RNA derived from mouse (coding region) of the receptor protein encoded by the cDNA fragment included in p3H2-17, PCR amplification was carried out In order to obtain a full-length open reading frame

disclosed, the following 4 primers were synthesized: Based on the nucleotide sequence of 3H2-17 which was (Nucleotide sequence of synthesized primer)

spleen and thymus was used.

5'-TAGTGTGTGGAGTCGTGTGGCTGGCTG-3'

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(SEQ ID NO: 20)

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5'-AGTCTTTGCTGCCACAGGCATCCAGCG-3'

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5'-CAAGCCAGTAAGGCTATGAAGGGCAGCAAG-3' (SEQ ID NO: 21)

☻ 5'-ACAGGACCTGCTGGCCATCCTGGCGACACA-3'

(SEQ ID NO: 22)

(SEQ ID NO: 23)

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of 5'Ampli Finder RACE kit from ClonTech Co. (ClonTech Co.). The 5'RACE was carried out according to the protocol

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cDNA thus prepared, the anchor and the aforementioned primer of poly(A) RNAs derived from mouse spleen and thymus by using attached to the 5'RACE kit. A mixture of a 1/200 amount of the the aforementioned primer  $oldsymbol{\oplus}$  and ligated with an anchor In an embodiment, cDNA was prepared from 2  $\mu$  g each

35 30 was subjected to PCR using 4 polymerases, Tag (Takara, Japan), Ex Tag (Takara, Japan), Vent (New England Biolabs) and Pfu electrophoresis and stained with ethidium bromide (EtBr). (Stratagene) under the following conditions: 96 °C for 30 sec., The results are shown in Figure 66. The amplified DNA band amount of the PCR product was subjected to agarose 60 °C for 60 sec., 72 °C for 90 sec. and 35 cycles. A 1/5

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appeared at an about 1 kbp position and the isolated about 1 kbp DNA band which was synthesized from poly(A) \*RNAs derived from mouse spleen and thymus by the 5'RACE using Ex Tag polymerase was treated with SUPREC \*TM\* 01 (Takara, Japan) to recover cDNA.

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The isolated DNA was subcloned into pCR <sup>TM</sup> I vector by using a TA Cloning Kit (Invitrogen Co.) and the vector was transfected into E. coli JM109 to obtain 3 transformant clones, N26, N64 and N75. The clone, N26, holds the thymusderived cDNA which is amplified by the 5'RACE and the clone, N75, holds the spleen-derived cDNA which is amplified by the 5'RACE (Figure 68).

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The 3'RACE was carried out according to the protocol of 3' RACE kit (GIBCO BRL Co.).

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In an embodiment, cDNA was prepared from 1  $\mu$  g each of poly(A) \*RNAs derived from mouse spleen and thymus by using an adaptor primer attached to the 3' RACE kit. A mixture of the adaptor primer thus prepared and a 1/10 amount of cDNA which was prepared by using the aforementioned primer (I) was subjected to 1st PCR using 4 polymerases, Tag (Takara, Japan), Vent (NEB) and Pfu (Stratagene) under the following conditions: 96 °C for 30 sec., 55 °C for 60 sec., 72 °C for 120 sec. and 30 cycles. A mixture of a 1/50 amount of the 1st PCR product, the aforementioned primer (I) and the adaptor primer was subjected to 2nd PCR using the aforementioned herein in the 5'RACE process. A 1/5 amount of the 2nd PCR product was subjected to agarose electrophoresis and stained with ethidium bromide. The results are shown in Figure 67.

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The amplified DNA band appeared at an about 1 kbp position (which was synthesized from poly(A) \*RNAs derived from mouse thymus by the 3'RACE using Vent polymerase) and the amplified DNA band appeared at an about 1 kbp position (which was synthesized from poly(A) \*RNAs derived from mouse thymus by the 3'RACE using Pfu polymerase) were treated with

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SUPREC  $^{TM}$  -01 (Takara, Japan) to recover cDNA, respectively.

The isolated DNAs were treated with T4
polynucleotide kinase (Wako Pure Chemical Co., Japan) to
add phosphate to the end thereof and the phosphorylated DNAs
were ligated with pUC18 Smal BAP (Pharmacia) by using DNA
Ligation Kit (Takara, Japan) followed by transformation of

E. coli JM109 to obtain 3 transformant clones, C2, C13 and
C15. The clones, C13 and C15, hold the thymus-derived cDNA
which is amplified by the 3'RACE and the clone, C2, holds the
thymus-derived cDNA which is amplified by the 3'RACE (Figure 68).

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Based on the nucleotide sequences of clones, N26, N64 and N75, which are considered to hold the N-terminal region of the open reading frame (ORF) of the cDNA fragment included in p3H2-17 and the nucleotide sequences of clones, C2, C13 and C15, which are considered to hold the C-terminal region of the open reading flame (ORF) of the cDNA fragment included in p3H2-17, the entire nucleotide sequence coding for the open reading flame and neighboring region of the receptor protein encoded by the cDNA included in p3H2-17 was determined. To be more specific, sequencing was carried out with the primers used in the 5'RACE and 3'RACE or synthetic primers for sequencing by using a DyeDeoxy Terminator Cycle Sequencing Kit (ABI Co.), the nucleotide sequences were decoded by using a fluorescent automatic sequencer. The obtained data of the DNA were analyzed by DNASIS (Hitachi System Engineering Co.,

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have been corrected by a way of thinking that, when nucleotides between two clones which are independently produced by PCR are identical (e.g. those between clones, N75 and N64, are identical) each other, the identical base is considered as correct. The determined nucleotide sequence is shown in Figure 69. The amino acid sequence is deduced based on the determined nucleotide sequence (Figure 69). Hydrophobicity plotting was carried out based on the deduced amino acid sequence (Figure 69). As a result, it was learned that it was a seven transmembrane G protein coupled receptor, as

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that it is homologous to mouse  $\mathbf{P}_{\mathbf{2}\mathbf{U}}$  purinoceptor and chicken it is suggested from the cDNA fragment included in p3H2-17. Homology retrieval at the amino acid level indicates

15 the open reading flame (ORF) was selected and used to construct P2Ypurinoceptor. N-terminal region of the receptor protein held by p3H2-17. DraIII and EcoRI, to obtain cDNA fragments which are the by the clone, N75, was digested with restriction enzymes, encoded by p3H2-17. In an embodiment, the cDNA fragment held plasmids carrying the full-length ORF of the receptor protein enzymes, DraIII and EcoRI. The N75-derived N-terminal cDNA fragment was obtained by the digestion of C13 with restriction regions from the DraIII site of the C-terminal and the long restriction enzymes, DraIII and EcoRI, to delete 5'-side The C-terminal cDNA fragment encoded by Cl3 was digested with transformant Escherichia coli JM109/pMAH2-17. DraIII-EcoRI fragment by using a DNA Ligation Kit (Takara, DraIII-EcoRI fragment was ligated with the long C13-derived Japan) and transfected into Escherichia coli JM109 to obtain Further, the clone which are free of an error in

2 Electrophysiological Measurement of Receptor Encoded by PMAH2-17

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Capping kit (Stratagene) to produce cRNA of this receptor the sequence thereof downstream from T7 promoter. The sites of pBluescript  $^{TM}$  II SK(+) (Stratagene) with directing receptor encoded by PMAH2-17 was inserted into the XhoI-XbaI electrophsiologically in Xenopus occytes. The ORF of the resulting plasmid as a template was treated with a mCAP  $\,$  m The receptor encoded by PMAH2-17 was examined

disclosed in Nathan Dascal et al., Proc. Natl. Acad. Sci. USA electrophysiological measurements. The measurement was carried incubated at 20 °C for 2 to 3 days and subjected to Vol..90, pp.6596-6600 (1993). The cRNA-injected oocytes were cRNA/50nl/oocyte), previously prepared according to the method The CRNA was injected into xenopus occytes (50ng

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clamping amplifier (CEz -/200, Nippon Koden, Co., Japan). and responses (current changes of the membrane) evoked by The initial membrane potential of oocytes was set to -60 mV amplifier (MEz-8300, Nippon Koden, Co., Japan), and a voltage al., Proc. Natl. Acad. Sci. USA, Vol. 90, pp.6596-6600 Array recorder, Nippon Koden, Co., Japan) (Nathan Dascal et addition of ligands were recorded with a recorder (Thermal out with a microelectrode-applicable high input resistance

phospholipase C-coupled receptors were observed in occytes injected with pMAH2-17 cRNA via stimulation by 10  $\mu$  M ATP ATP stimulation. in oocytes injected with  ${\rm H_2O}$ , instead of pMAH2-17 cRNA, by the (Figure 75). In contrast, such a current was not observed Typical inward currents elicited upon activation of

encoded by pMAH2-17 cRNA is classified into a subtype within the ATP receptor, P2 purinoceptor. In conclusion, it is considered that the receptor

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### Example 20

Cloning of Rabbit Gastropyrolic Part Smooth Muscle-Derived G (1) Preparation of Poly(A) RNA Fraction from Rabbit Protein Coupled Receptor Protein cDNA Gastropyrolic Part Smooth Muscle and Synthesis of cDNA

30 25 **3**5 part smooth muscles by the guanidine thiocyanate method poly(A) RNA fraction was added a random DNA hexamer (BRL Co.) purifying kit (Pharmacia Co.). Next, to 5  $\mu$  g of the then, poly(A) RNA fractions were prepared with a mRNA (Kaplan B.B. et al., Biochem. J. 183, 181-184 (1979)) and, as a primer, and the resulting mixture was subjected to ethanol, and was then dissolved in 30  $\mu$  1 of TE. was extracted with phenol/chloroform (1:1), precipitated in to synthesize complementary DNAs. The reaction product buffer attached to the MMLV reverse transcriptase kit reaction with MMLV reverse transcriptase (BRL Co.) in the A total RNA was prepared from rabbit gastropyrolic

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(2) Amplification of Receptor cDNA by PCR Using Rabbit Gastropyrolic Part Smooth Muscle-Derived cDNA and Sequencing By using, as a template, 1  $\mu$  1 of cDNA prepared from the rabbit gastropyrolic part smooth muscle in the above step (1), PCR amplification using the DNA primer having a nucleotide sequence represented by SEQ ID NO: 10 and the DNA primer having a nucleotide sequence represented by SEQ ID NO: 4 synthesized in Example 15 was carried out.

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A reaction solution was composed of the synthetic DNA primers (SEQ: 5' primer sequence and 3' primer sequence) each in an amount of 100 pM, 0.25 mM dNTPs, 1  $\mu$  1 of Tag DNA polymerase and 10  $\mu$  1 of buffer attached to the enzyme kit, and the total amount of the reaction solution was made to be 100 $\mu$  1. The cycle for amplification including 96 °C for 30 sec., 45 °C for 1 min. and 60 °C for 3 min. was repeated 25 times by using a Thermal Cycler (Perkin-Elmer Co.). The amplified products were confirmed relying upon 1.2% agarose gel electrophoresis and ethidium bromide staining.

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(3) Subcloning of PCR Product into Plasmid Vector and Selection of Novel Receptor Candidate Clone via Decoding Nucleotide Seguence of Inserted cDNA Region

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The PCR products obtained in the above step (2) were separated by using a 1.0% low-melting temperature agarose gel, the band parts were excised from the gel with a razor blade, and were electro-eluted, extracted with phenol and precipitated in ethanol to recover DNAs. According to the Protocol attached to a TA Cloning Kit (Invitrogen Co.), the recovered DNAs were subcloned to the plasmid vector, pcr II. The recombinant vectors were introduced into E. Coli JM109 competent cells (Takara Shuzo Co., Japan) to produce transformants. Then, transformant clones having a CDNA-inserted fragment were selected in an LB agar culture medium containing ampicillin, IPTG and X-gal. Only transformant clones exhibiting white color were picked with a sterilized toothstick to obtain 100 transformant clones.

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The individual clones were cultured overnight in an LB culture medium containing ampicillin and treated with the automatic plasmid extracting machine PI-100 (Kurabo Co., Japan) to prepare plasmid DNAs. An aliquot of the DNAs thus prepared was cut by EcoRI to confirm the size of the cDNA fragment that was inserted. An aliquot of the remaining DNAs was further processed with RNase, extracted with phenol/chloroform, and precipitated in ethanol so as to be condensed. Sequencin was carried out by using a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNAs were decoded by using a fluorescent automatic sequencer.

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amino acid sequence level to find a novel receptor protein which are shown in Figures 71 and 72. To further confirm this fact, sensitive opsin receptor protein (A03156). The aforementioned JM109/pMN128. The nucleotide sequences of the cDNA fragments nucleotide sequences were converted into amino acid seguences [Figure 71 and Figure 72], and homology retrieval was carried out in view of hydrophobicity plotting [Figure 73] and at the determined nucleotide sequence. As a result, it was learned receptor protein (A03159), 20% homology relative to rat-deri bradykinin receptor (type  $\mathrm{B}_{2}$ ) protein (A41283), 24% homology has 27% homology relative to hamster-derived  $\,eta_{_{\! 4}}$ -adrenaline when they are registered as data to NBRF-PIR and are usually Homology retrieval was carried out based upon the by using DNASIS (Hitachi System Engineering Co., Japan) the abbreviations in parentheses are reference numbers assigned (S11377) and 23% homology relative to human-derived blue relative to human-derived dopamine  $\mathtt{D}_1$  receptor protein plasmid possessed by the transformant Escherichia coli that a novel G protein coupled receptor protein was been encoded by the CDNA fragment insert in the

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called "Accession Numbers".

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### Example 21

Cloning of cDNA Comprising Whole Coding Regions for Receptor Protein from Human-Derived DNA Library

The DNA library constructed by Clontech wherein \$\frac{1}{2}\$ \$\lambda\$ gtll phage vector is used (CLONTECH Laboratories, Inc.; CLH L1008b) was employed as a human placenta-derived cDNA library. The human placenta cDNA library (1 x 10 pfu (plaque forming units)) was thermally denatured. By using the human placenta-derived cDNA library, PCR amplification using the DNA primer having a nucleotide sequence represented by SEQ ID NO: 20 and the DNA primer having a nucleotide sequence represented by SEQ ID NO: 23 synthesized in Example 19 was carried out.

(Nucleotide sequence of synthesized primer)

15 D 5'-TAGTGTGGGAGTCGTGGCTGGCTG-3'

(SEQ ID NO: 20)

② 5'-ACAGGACCTGCTGGGCCATCCTGGCGACACA-3'

(SEQ ID NO: 23)

The isolated DNA was subcloned using a TA Cloning Kit (Invitrogen Co.) and sequencing was carried out. Figure 76 shows a nucleotide sequence of obtained cDNA fragment, ph3H2-17. As a result, it was learned that ph3H2-17 is highly homologous to the mouse purinoceptor cDNA fragment, p3H2-17. It is strongly suggested that the human-derived cDNA fragment is a partial nucleotide sequence of human purinoceptor.

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Based on the nucleotide sequence of ph3H2-17 which was sequenced, the following 2 primers were synthesized:

(Nucleotide sequence of synthesized primer)

(Nucleotide sequence of synthesized primer)

③ 5'-ACAGCCATCTTCGCTGCCACAGGCAT-3'

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(SEQ ID NO: 58)

⑤ 5'-AGACAGTAGCAGGCCAGCAGGGCAAA-3'

(SEQ ID NO: 59)

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The above synthetic 2 primers were each used in combination with  $\lambda$  gt 11 primers (Takara, Japan; catalogue 3864) for obtaining full-length human prinoceptor cDNA. Thus, using thermally denatured, human placenta-derived  $\lambda$  gt 11 cDNA libraries (CLONTECH; CLHL 1008b), first RCR amplification using

v

a combination of the DNA primer having a nucleotide sequence

represented by SEQ ID NO: 20 with  $\lambda$  gt 11 Forward primer, of the DNA primer having a nucleotide sequence represented by SEQ ID NO: 20 with  $\lambda$  gt 11 Reverse primer, of the DNA primer having a nucleotide sequence represented by SEQ ID NO: 23 with  $\lambda$  gt 11 Forward primer, and of the DNA primer having a nucleotide sequence represented by SEQ ID NO: 23 with  $\lambda$  gt 11 Reverse primer was carried out with Ex Tag polymerase (Takara, Japan) (30 cycles; 95°C/30 seconds, 55°C/60 seconds, and 72°C/60 seconds), respectively.

Next, by using a 1/50 of the 1st PCR product, second RCR amplification was carried in the same manner as in the first PCR except for using the DNA primer having a nucleotide sequence represented by SEQ ID NO: 58 in place of SEQ ID NO: 20 and the DNA primer having a nucleotide sequence represented by SEQ ID NO: 59 in place of SEQ ID NO: 23 (30 cycles; 95°C/30 seconds, 65°C/60 seconds and 72°C/60 seconds). The amplified product DNA was subcloned using a TA Cloning Kit (Invitrogen Co.) and sequencing was carried out for three clones each of 5' and 3' sides (Figure 77).

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Based on the amino acid sequence (Figure 77) deduced from the determined nucleotide sequence of human purinoceptor cDNA as shown in Figure 77, hydrophobicity plotting was carried out (Figure 78). As a result, it was learned that the human-derived receptor is a novel seven transmembrane G protein coupled receptor, similarly to the mouse type. It was also learned that the deduced amino acid sequence of human receptor has 87% homology relative to the amino acid sequence of mouse purinoceptor and its amino acid residues are well conserved

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Clones free of PCR errors which often occur in a PCR amplification were selected and restriction enzyme regions

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reading frame of human purinoceptor cDNA. The plasmid phAH2-17 The restriction enzyme regions thus obtained were subjected to is possessed by transformant Escherichia coli JM109/phAH2-17. construction of plasmid phAH2-17 having a full-length open comprising overlapping areas were obtained therefrom.

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efficient amplification of DNAs that encode G protein coupled receptor proteins. This makes it possible to efficiently screen for the DNAs coding for G protein caupled receptor The DNA primers of the present invention allow proteins and to accomplish the cloning.

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The G protein coupled receptor protein of the present invention and their G protein coupled receptor protein-encoding DNA are advantageously useful in:

- determining ligands, Θ
- obtaining antibodies and an antisera, 0 15
- constructing systems for expressing recombinant receptor
- investigating or developing receptor-binding assay systems and screening for pharmaceutical candidate compounds, by using the above expression system ✐

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- designing drugs based upon comparisons with ligands and receptors having a structure similar or analogous thereto, ഇ
- preparing probes and/or PCR primers in gene diagnosis, and gene manipulating therapy.

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development of unique pharmaceuticals acting upon these systems. properties of the G protein coupled receptor will lead to the In particular, discovering the structure and

within the skill of the art. All patents, patent applications, and publications mentioned herein, both supra and infra, are The practice of the present invention will employ, otherwise indicated, conventional techniques of molecular immunology, bioscience, and medical technology, which are biology, microbiology, recombinant DNA, pharmacology, hereby incorporated herein by reference.

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### SEQUENCE LISTING

### (1) GENERAL INFORMATION:

(i) APPLICANT:

- Takeda Chemical Industries, Ltd. 1-1, Doshomachi 4-chome, Chuo-ku (A) NAME:
  - STREET: <u>@</u>
    - Osaka-shi ပ္
      - Osaka STATE: 9
- COUNTRY: Japan Θ)
- 541 (F) POSTAL CODE (ZIP):
- (ii) TITLE OF INVENTION: G Protein Coupled Receptor Protein, Production, And Use Thereof
- (iii) NUMBER OF SEQUENCES:

61

- (iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
  (B) COMPUTER: IBM PC compatible
  (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  (D) SOFTWARE: Patentin Release #1.0, Version #1.25 (EPO)
- (2) INFORMATION FOR SEQ ID NO: 1:
- SEQUENCE CHARACTERISTICS: <u>:</u>
  - (A) LENGTH: (B) TYPE:
- Nucleic acid STRANDEDNESS: Single

  - Linear (D) TOPOLOGY:
- Other nucleic acid (ii) MOLECULE TYPE:
- Synthetic DNA

N is A, G, C, or T

(iii) FEATURES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

25

(2) INFORMATION FOR SEQ ID NO: 2:

CGTGGSCMTS STGGGCAACN YCCTG

- SEQUENCE CHARACTERISTICS: <u>:</u>
  - (A) LENGTH: (B) TYPE:
- Nucleic acid Single STRANDEDNESS:
  - Linear (C) STRANDEDNE: (D) TOPOLOGY:
- Other nucleic acid Synthetic DNA (ii) MOLECULE TYPE:
- (iii) FEATURES:
- N is A, G, C, or T
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

27 GTINGWRRGGC ANCCAGCAGA KGGCAAA

```
(2) INFORMATION FOR SEQ ID NO: 3:
                          (ii) MOLECULE TYPE: Other nucleic acid
                                                                                                              (i) SEQUENCE CHARACTERISTICS:
(iii) FEATURES:
                                                 (D) TOPOLOGY:
                                                              (C) STRANDEDNESS: Single
                                                                                (B) TYPE:
                                                                                               (A) LENGTH:
                                                                                   Nucleic acid
                                                                                                                                                                                                          -239-
    N is inosine
                   Synthetic DNA
```

CTCGCSGCYM TNRGYATGGA YCGNTAT

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS: (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear (B) TYPE: (A) LENGTH: Nucleic acid

(ii) MOLECULE TYPE: Other nucleic acid Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4: (iii) FEATURES: N is inosine

CATGIRGWAG GGAANCCAGS AMANRARRAA

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS: (B) TYPE: Nucleic (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear (A) LENGTH: Nucleic acid

(ii) MOLECULE TYPE: Other nucleic acid Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CTGACYGYTC THRSHRYTGA CMGVTAC

(i) SEQUENCE CHARACTERISTICS:

(2) INFORMATION FOR SEQ ID NO: 6:

(B) TYPE: Nucleic (C) STRANDEDNESS: Single (A) LENGTH: 2/
Nucleic acid

> (D) TOPOLOGY: Linear - 2 4 0 -

(ii) MOLECULE TYPE: Other nucleic acid Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6: (iii) FEATURES: N is inosine

CTGACYGYTC THRSHRYTGA CMGVTAT (2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(C) STRANDEDNESS: Single (B) TYPE: (A) LENGTH: Nucleic acid

(D) TOPOLOGY:

(ii) MOLECULE TYPE: Other nucleic acid Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CTCGCSGCYM TNRGYATGGA YCGNTAC

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS: (A) LENGTH:

(B) TYPE: (C) STRANDEDNESS: Single Nucleic acid

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

(2) INFORMATION FOR SEQ ID NO: 9:

GATGTGRTAR GGSRNCCAAC AGANGRYAAA

S O

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: Nucleic acid

(B) TYPE: Nucleic (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid Synthetic DNA

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N is inosine

(iii) FEATURES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GATGTGRIAR GGSRNCCAAC AGANGRYGAA

(2) INFORMATION FOR SEQ ID NO: 10:

SEQUENCE CHARACTERISTICS: (ĭ)

Nucleic acid (A) LENGTH: 27
(B) TYPE: Nucleic (C) STRANDEDNESS: Single

Linear (D) TOPOLOGY:

Other nucleic acid Synthetic DNA (ii) MOLECULE TYPE:

N is inosine (iii) FEATURES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GYCACCAACN WSTTCATCCT SWNHCTG

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS: (A) LENGTH:

(B) TYPE:

Nucleic acid STRANDEDNESS: Single

(C) STRANDEDNES (D) TOPOLOGY:

Linear

Other nucleic acid Synthetic DNA (ii) MOLECULE TYPE:

N is inosine (iii) FEATURES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

ASNSANRAAG SARTAGANGA NRGGRTT

(2) INFORMATION FOR SEQ ID NO: 12:

SEQUENCE CHARACTERISTICS: (A) LENGTH: 25 <u>:</u>

Nucleic acid Single (C) STRANDEDNESS:

Linear (D) TOPOLOGY;

Other nucleic acid Synthetic DNA (ii) MOLECULE TYPE:

N is inosine (iii) FEATURES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

TGNTSSTKMT NGSNGTKGTN GGNAA

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(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: (B) TYPE:

Nucleic acid

(C) STRANDEDNESS: Single (D) TOPOLOGY: Linear

Other nucleic acid (ii) MOLECULE TYPE:

Synthetic DNA

N is inosine (iii) FEATURES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13;

AYCKGTAYCK GTCCANKGWN ATKGC

(2) INFORMATION FOR SEQ ID NO: 14:

Nucleic acid

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic ac
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

Linear

Other nucleic acid Synthetic DNA (ii) MOLECULE TYPE:

N is inosine (iii) FEATURES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

CATKKCCSTG GASAGNTAYN TRGC

(2) INFORMATION FOR SEQ ID NO: 15:

SEQUENCE CHARACTERISTICS: (ï)

(A) LENGTH:

Nucleic acid (B) TYPE:

STRANDEDNESS: Single (C) STRANDEDNES (D) TOPOLOGY:

Other nucleic acid Synthetic DNA (ii) MOLECULE TYPE:

N is inosine (iii) FEATURES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GWWGGGSAKC CAGCASANGG CRAA

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(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS: (A) LENGTH:

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(ii) MOLECULE TYPE:

Other nucleic acid

Synthetic DNA

(D) TOPOLOGY:

Linear

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(2) INFORMATION FOR SEQ ID NO: 18:
                                                                                                                                                                                                                                                                                    NGGNANCCAR CANANNRNRA A
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             ARYYTNGCNN TNGCNGAY
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    (2) INFORMATION FOR SEQ ID NO: 17:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:
                                       (iii) FEATURES:
                                                                                             (ii) MOLECULE TYPE:
                                                                                                                                                                                                        (i) SEQUENCE CHARACTERISTICS:
                                                                                                                                                                                                                                                                                                                 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:
                                                                                                                                                                                                                                                                                                                                                                                                                                                   (ii) MOLECULE TYPE:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              (1) SEQUENCE CHARACTERISTICS:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      (iii) FEATURES: 15th N is A, G, C, or T
6th, 9th, 10th & 12th Ns are inosine
                                                                                                                                                                                                                                                                                                                                                                                             (iii) FEATURES:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         (ii) MOLECULE TYPE:
                                                                                                                            (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear
                                                                                                                                                                       (B) TYPE:
                                                                                                                                                                                     (A) LENGTH:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      (A) LENGTH: 21
(B) TYPE: Nucleic
(C) STRANDEDNESS: Single
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         (D) TOPOLOGY:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             (B) TYPE: Nucleic (C) STRANDEDNESS: Single
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               (D) TOPOLOGY:
                                     N is inosine
                                                                                         Other nucleic acid
                                                                           Synthetic DNA
                                                                                                                                                                    Nucleic acid
                                                                                                                                                                                                                                                                                                                                                13th, 15th, 16th & 18th Ns are each A, G, C, or T lst, 4th, 6th Ns are inosine
                                                                                                                                                                                                                                                                                                                                                                                                                                               Other nucleic acid
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Other nucleic acid Synthetic DNA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Linear
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Nucleic acid
                                                                                                                                                                                                                                                                                                                                                                                                                                 Synthetic DNA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Linear
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Nucleic acid
```

GCCTSNTNRN SATGWSTGTG GANMGNT (2) INFORMATION FOR SEQ ID NO: 19:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH:

(B) TYPE: Nucleic (C) STRANDEDNESS: Single

Nucleic acid

CAAGCCAGTA AGGCTATGAA GGGCAGCAAG

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

(ii) MOLECULE TYPE:

Other nucleic acid

Synthetic DNA

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30
(B) TYPE: Nucleic
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

Nucleic acid

(2) INFORMATION FOR SEQ ID NO: 22: AGTCTTTGCT GCCACAGGCA TCCAGCG TAGTGTGTGG AGTCGTGTGG CTGGCTG (2) INFORMATION FOR SEQ ID NO: 21: GAWSNIGMYN ANRIGGWAGG GNANCCA (2) INFORMATION FOR SEQ ID NO: 20: (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21: (ii) MOLECULE TYPE: (i) SEQUENCE CHARACTERISTICS: (ii) MOLECULE TYPE: (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20: (i) SEQUENCE CHARACTERISTICS: (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19: (iii) FEATURES: (B) TYPE: Nucleic (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear (A) LENGTH: (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear (B) TYPE: LENGIH: Other nucleic acid Nucleic acid Other nucleic acid Synthetic DNA Synthetic DNA Nucleic acid N is inosine

- (2) INFORMATION FOR SEQ ID NO: 23:
- SEQUENCE CHARACTERISTICS:
- Nucleic acid
- (A) LENGTH: 31
  (B) TYPE: Nucleic (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear
- Other nucleic acid Synthetic DNA (ii) MOLECULE TYPE:
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

ACAGGACCTG CTGGGCCATC CTGGCGACAC A

- (2) INFORMATION FOR SEQ ID NO: 24:
- (1) SEQUENCE CHARACTERISTICS: (A) LENGTH: 91
- Amino acid
  - Linear (C) TOPOLOGY:
- Peptide (ii) MOLECULE TYPE:
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:
- Leu Val Leu Val Ile Ala Arg Val Arg Arg Leu His Asn Val Thr Asn

Phe Leu Ile Gly Asn Leu Ala Leu Ser Asp Val Leu Met Cys Thr Ala  $20 \ 25$ 

Phe Gly Gly Gly Leu Cys His Leu Val Phe Phe Leu Gln Pro Val Thr 50 55 Val Tyr Val Ser Val Phe Thr Leu Thr Thr Ile Ala Val Asp Arg Tyr

- Val Val Leu Val His Pro Leu Arg Arg Ile 85
- (2) INFORMATION FOR SEQ ID NO: 25:
- (i) SEQUENCE CHARACTERISTICS:
- Amino acid (A) LENGTH:
  - (C) TOPOLOGY:
- Peptide (ii) MOLECULE TYPE:
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Gly Leu Leu Leu Val Thr Tyr Leu Leu Pro Leu Leu Val Ile Leu Leu

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Ser Tyr Val Arg Val Ser Val Lys Leu Arg Asn Arg Val Val Pro Gly 72

Cys Val Thr Gln Ser Gln Ala Asp Trp Asp Arg Ala Arg Arg Arg Arg  $^{45}$ 

Thr Phe Cys Leu Leu Val Val Val Val Val Val 50

- (2) INFORMATION FOR SEQ ID NO: 26:
- (i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 370
  (B) TYPE: Amino ac
- Amino acid
  - Linear
  - (C) TOPOLOGY:
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Peptide

(ii) MOLECULE TYPE:

Met Ala Ser Ser Thr Thr Arg Gly Pro Arg Val Ser Asp Leu Phe Ser  $_{\rm 1}$ 

Gly Leu Pro Pro Ala Val Thr Thr Pro Ala Asn Gln Ser Ala Glu Ala  $20\ 20\$ 

Ser Ala Gly Asn Gly Ser Val Ala Gly Ala Asp Ala Pro Ala Val Thr 35 Pro Phe Gln Ser Leu Gln Leu Val His Gln Leu Lys Gly Leu Ile Val 50 60

Leu Leu Tyr Ser Val Val Val Val Gly Leu Val Gly As<br/>n Cys Leu  $65 \ \ \,$ 

Leu Val Leu Val Ile Ala Arg Val Arg Arg Leu His Asn Val Thr Asn 85 95 Phe Leu 11e Gly Asn Leu Ala Leu Ser Asp Val Leu Met Cys Thr Ala 100

Cys Val Pro Leu Thr Leu Ala Tyr Ala Phe Glu Pro Arg Gly Trp Val  $115\,$ 

Phe Gly Gly Gly Leu Cys His Leu Val Phe Phe Leu Gln Pro Val Thr 130

Val Tyr Val Ser Val Phe Thr Leu Thr Thr Ile Ala Val Asp Arg Tyr 145

Val Val Leu Val His Pro Leu Arg Arg 11e Ser Leu Arg Leu Ser 175  $175\,$ 

Ala Tyr Ala Val Leu Ala Ile Trp Ala Leu Ser Ala Val Leu Ala Leu

Pro Ala Ala Val His Thr Tyr His Val Glu Leu Lys Pro His Asp Val  $195\,$   $200\,$ 

Arg Leu Cys Glu Glu Phe Trp Gly Ser Gln Glu Arg Gln Arg Gln Leu  $210 \ \ 215 \ \ 220$ 

Ile Leu Leu Ser Tyr Val Arg Val Ser Val Lys Leu Arg Asn Arg Val
250
255 Tyr Ala Trp Gly Leu Leu Leu Val Thr Tyr Leu Leu Pro Leu Leu Val  $225 \ 230 \ 235 \ 240$ 

Val Pro Gly Cys Val Thr Gln Ser Gln Ala Asp Trp Asp Arg Ala Arg 260 265 270

Arg Arg Thr Phe Cys Leu Leu Val Val Val Val Val Phe Ala 275 280 285

Val Cys Trp Leu Pro Leu His Val Phe Asn Leu Leu Arg Asp Leu Asp 290 295 300

His Trp Leu Ala Met Ser Ser Ala Cys Tyr Asn Pro Phe Ile Tyr Ala 325 330 Pro His Ala Ile Asp Pro Tyr Ala Phe Gly Leu Val Gln Leu Leu Cys 305 315 320

Trp Leu His Asp Ser Phe Arg Glu Glu Leu Arg Lys Leu Leu Val Ala 340 345

Trp Pro Arg Lys Ile Ala Pro His Gly Gln Asn Met Thr Val Ser Val  $355 \ \ 360 \ \ 365$ 

Val Ile 370

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 206

(B) TYPE: Amino ac

(C) TOPOLOGY: Linear

Amino acid

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Leu Val Leu Val Ile Ala Arg Val Arg Arg Leu Tyr Asn Val Thr Asn 1 5 10

Phe Leu Ile Gly Asn Leu Ala Leu Ser Asp Val Leu Met Cys Thr Ala 20. 25 30

Cys Val Pro Leu Thr Leu Ala Tyr Ala Phe Glu Pro Arg Gly Trp Val

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Val Tyr Val Ser Val Phe Thr Leu Thr Thr Ile Ala Val Asp Arg Tyr 65 70 75 80 Phe Gly Gly Gly Leu Cys His Leu Val Phe Phe Leu Gln Ala Val Thr  $50 \ \ 55 \ \ 60$ 

Val Val Leu Val His Pro Leu Arg Arg Arg Ile Ser Leu Arg Leu Ser  $90\,$  95

Ala Tyr Ala Val Leu Ala Ile Trp Val Leu Ser Ala Val Leu Ala Leu 100 105 110

Arg Leu Cys Glu Glu Phe Trp Gly Ser Gln Glu Arg Gln Arg Gln Leu 130 135 140 Pro Ala Ala Val His Thr Tyr His Val Glu Leu Lys Pro His Asp Val 115 120

Tyr Ala Trp Gly Leu Leu Val Thr Tyr Leu Leu Pro Leu Leu Val 145 150 155

Ile Leu Leu Ser Tyr Ala Arg Val Ser Val Lys Leu Arg Asn Arg Val 170 175

Val Pro Gly Arg Val Thr Gln Ser Gln Ala Asp Trp Asp Arg Ala Arg 180 185

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 126

(B) TYPE: (C) TOPOLOGY: Amino acid

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Ser Leu Cys Glu Glu Phe Trp Gly Ser Gln Glu Arg Gln Arg Gln Ile  $50\,$ Pro Ala Ala Val His Thr Tyr His Val Glu Leu Lys Pro His Asp Val  $35 \ \ \, 40 \ \ \,$ Val Val Leu Val His Pro Leu Arg Arg Arg Ile Ser Leu Arg Leu Ser 1 15 Ala Tyr Ala Val Leu Gly Ile Trp Ala Leu Ser Ala Val Leu Ala Leu 20 25 30

Tyr Ala Trp Gly Leu Leu Gly Thr Tyr Leu Leu Pro Leu Leu Ala

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55 70 75	80	
ile Leu Leu Ser Tyr Val Arg Val Ser Val Lys 85	Leu Arg Asn Arg Val 95	
Val Pro Gly Ser Val Thr Gln Ser Gln Ala Asp 105	Trp Asp Arg Ala Arg	
Arg Arg Arg Thr Phe Cys Leu Leu Val Val Val Val 115	. Val Val 125	
(2) INFORMATION FOR SEQ ID NO: 29:		
(i) SEQUENCE CHARACTERISTICS: (A) LENCTH: 273 (B) TYPE: Nucleic acid (C) STRANDEDNESS: Double (D) TOPOLOGY: Linear		
(ii) MOLECULE TYPE: CDNA		
(ix) FEATURE (C) IDENTIFICATION METHOD: S		
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:	: 29:	
CTGGTGCTGG TGATCGCGCG GGTGCGCCGG CTGCACAACG TGACGAACTT CCTCATCGGC	s tgacgaactt cctcatcggc 60	
AACCTGGCCT TGTCCGACGT GCTCATGTGC ACCGCCTGCG TGCCGCTCAC GCTGGCCTAT	G TGCCGCTCAC GCTGGCCTAT 120	
GCCTTCGAGC CACGCGGCTG GGTCTTCGGC GGCGGCCTGT GCCACCTGGT	T GCCACCTGGT CTTCTTCCTG 180	
CAGCCGGTCA CCGTCTATGT GTCGGTGTTC ACGCTCACCA CCATCGCAGT GGACCGGTAC	A CCATCGCAGT GGACCGGTAC 240	
GICGIGCIGG IGCACCCGCI GAGGCGGCGC AIC	273	
(2) INFORMATION FOR SEQ ID NO: 30:		

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ë
ü
SEQ
FOR
INFORMATION
(2)

HARACTERISTICS:	1110
SEQUENCE CHARA	E
(;)	ì

(B) TYPE: Nucleic acid (C) STRANDEDNESS: Double (D) TOPOLOGY: Linear

CDNA (ii) MOLECULE TYPE: (ix) FEATURE (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

60	120	180	240	300	360	420	480	540	900	٥	120	780	840	900	960	1020	1080
GCTGCCGCCG	Greenseer	TCAGCTGAAG	CAACTGCCTG	CCTCATCGGC	GCTGGCCTAT	crrcrrccrs	GGACCGCTAC	CTACGCTGTG	CACCTATCAC	CCAGGAGCGÚ	Tercerecte	GCCGGGCTGC	crecrrecre	CAACCTGCTG	GCTGCTCTGC	GCTGCACGAC	AGCCCCCCAT
ATGCCCTCAT CGACCACTCG GGCCCCCAGG GTTTCTGACT TATTTTCTGG GCTGCCGCCG	GCGGTCACAA CTCCCGCCAA CCAGAGCGCA GAGGCCTCGG CGGGCAACGG GTCGGTGGCT	GECECGGACE CICCAGCCGI CACGCCCTIC CAGAGCCTGC AGCTGGTGCA TCAGCTGAAG	GGGCTGATCG TGCTGCTCTA CAGCGTCGTG GTGGTCGTGG GGCTGGTGGG CAACTGCCTG	CIGGIGCIGG IGATCGCGCG GGIGCCGC CIGCACAACG IGACGAACTI CCICATCGGC	AACCTGGCCT TGTCCGACGT GCTCATGTGC ACCGCCTGCG TGCCGCTCAC GCTGGCCTAT	GCCACCTGGT	CAGCCGGTCA CCGTCTATGT GTCGGTGTTC ACGCTCACCA CCATCGCAGT GGACCGCTAC	GICGIGCIGG IGCACCCGCI GAGGCGGCGC AICTCGCIGC GCCICAGCGC CIACGCIGIG	CTGGCCATCT GGGGGTGTC CGCGGTGCTG GCGCTGCCG CCGCCGTGCA CACCTATCAC	GIGGAGCICA AGCCGCACGA CGIGCGCCIC IGCGAGGAGI ICTGGGGCIC CCAGGAGCG	CAGCGCCAGC TCTACGCCTG GGGGCTGCTG CTGGTCACCT ACCTGCTCCC TCTGCTGGTC	CTTACGTCCG GGTGTCAGTG AAGCTCCGCA ACCGCGTGGT GCCGGGCTGC	GTGACCCAGA GCCAGGCCGA CTGGGACGGC GCTCGGCGCC GGCGCACCTT CTGCTTGCTG	GTECTICATICS TECTICATE TECCTECTEC TECACGTET CAACCTECTE	CECEACOTOS ACCOCCACOS CATOGACOCT TACECOTTTE GEOTGETECA GOTGOTOTEC	CACTGGCTCG CCATGAGTTC GGCCTGCTAC AACCCCTTCA TCTACGCCTG GCTGCACGAC	AGCTICCGCG AGGAGCIGCG CAAACIGIIG GICGCITGGC CCCGCAAGAI AGCCCCCAI
GITICIGACT	GAGGCCTCGG	CAGAGCCTGC	стестсетес	CTGCACAACG	ACCECCTECE	GCCGCCTGT	ACGCTCACCA	ATCTCGCTGC	GCGCTGCCCG	TGCGAGGAGT	CTGGTCACCT	AAGCTCCGCA	GCTCGGCGCC	recereces	TACGCCTTTG	AACCCCTTCA	GTCGCTTGGC
GGCCCCAGG	CCAGAGCGCA	CACGCCCTTC	CAGCGTCGTG	встесессве	GCTCATGTGC	GGTGTTCGGC	GTCGGTGTTC	GAGGCGGCGC	ceceeneere	cerececere	GGGCCTGCTG	GGTGTCAGTG	CTGGGACCGC	ceccercrec	CATCGACCCT	GCCTGCTAC	CAAACTGTTG
CGACCACTCG	CTCCCGCCAA	CTCCAGCCGT	recrectera	TGATCGCGCG	TGTCCGACGT	GCCTTCGAGC CACGCGGCTG GGTGTTCGGC GGCGGCCTGT GCCACCTGGT	CCGTCTATGT	TGCACCCGCT	GGGCGCTGTC	AGCCGCACGA	TCTACGCCTG		GCCAGGCCGA	TGGTGGTGTT	ACCCCCACGC	CCATGAGTTC	AGGAGCTGCG
 ATGCCCTCAT	GCGGTCACAA	GCCCCGACG	GGCTGATCG	crecrecres	AACCTGGCCT	GCCTTCGAGC	CAGCCGGTCA	Greerscree	CTGGCCATCT	GTGGAGCTCA	CAGCGCCAGC	ATCCTCCTGT	GTGACCCAGA	GTGGTGGTCG	CGGGACCTCG	CACTGGCTCG	AGCTTCCGCG

(2) INFORMATION FOR SEQ ID NO: 32:

GIGICAGIGA AGCICCGCAA CEGCGIGGIG CCGGGCTGCG TGACCCAGAG CCAGGCCGAC 120

TGGSACCGCG CTCGCCGCCG GCGCACCTTC TGCTTGCTGG TGGTGGTCGT GGTGGTG

GECCTGCTGC TGGTCACCTA CCTGCTCCTT CCTCCTGTC TTACGTCCGG 60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

(ix) FEATURE (C) IDENTIFICATION METHOD: S

CDNA

(ii) MOLECULE TYPE:

(A) LENGTH: 177
(B) TYPE: Nucleic acid (C) STRANDEDNESS: Double (D) TOPOLOGY: Linear

SEQUENCE CHARACTERISTICS:

(i)

177

GGCCAGAATA TGACCGTCAG CGTGGTCATC

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(i) SEQUENCE CHARACTERISTICS: (A) LENGTH:

(B) TYPE: Nucleic(C) STRANDEDNESS: Double(D) TOPOLOGY: Linear Nucleic acid

(ii) MOLECULE TYPE: CDNA

(ix) FEATURE (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

CTGGTGCTGG TGATCGCGCG GGTGCGCCCGG CTGTACAACG TGACGAATTT CCTCATCGGC AACCTGGCCT TGTCCGACGT GCTCATGTGC ACCGCCTGCG TGCCGCTCAC GCTGGCCTAT 120 CAGGCGGTCA CCGTCTATGT GTCGGTGTTC ACGCTCACCA CCATCGCAGT GGACCGCTAC 240 GCCTTCGAGC CACGCGGCTG GGTGTTCGGC GGCGGCCTGT GCCACCTGGT CTTCTTCCTG GTGGAGCTCA AGCCGCACGA CGTGCGCCTC TGCGAGGAGT TCTGGGGCTC CCAGGAGCGC 420 GTCGTGCTGG TGCACCCGCT GAGGCGGCGC ATCTCGCTGC GCCTCAGCGC CTACGCTGTG 300 GTGACCCAGA GCCAGGCCGA CTGGGACCGC GCTCGGCGCC GGCGCACCTT CTGCTTGCTG 600 ATCCTCCTGT CTTACGCCCG GGTGTCAGTG AAGCTCCGCA ACCGCGTGGT GCCGGGCCGC 540 CAGCGCCAGC TCTACGCCTG GGGGCTGCTG CTGGTCACCT ACCTGCTCCC TCTGCTGGTC 480 CTGGCCATCT GGGTGCTGTC CGCGGTGCTG GCGCTGCCC CCGCCGTGCA CACCTATCAC 360 GIGGIGGICG IGGIGGIG

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:

(B) TYPE: Nucleic (C) STRANDEDNESS: Double (D) TOPOLOGY: Linear Nucleic acid

(ii) MOLECULE TYPE: CDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO: 33:

CTGGGCATCT GGGCTCTATC TGCAGTGCTG GCGCTGCCGG CCGCGGTGCA CACCTACCAT GIGGIICIGG IGCACCCGCI ACGICGGCGC AITICACIGA GGCICAGCGC CIACGCGGIG 6

GTGGAGCTCA AGCCCCACGA CGTGAGCCTC TGCGAGGAGT TCTGGGGCTC GCAGGAGCGC

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CAACGCCAGA TCTACGCCTG GGGGCTGCTT CTGGGCACCT ATTTGCTCCC CCTGCTGGCC GTGACCCAGA GTCAAGCTGA CTGGGACCGA GCGCGTCGCC GCCGCACTTT CTGTCTGCTG ATCCTCCTGT CTTACGTACG GGTGTCAGTG AAGCTGAGGA ACCGCGTGGT GCCTGGCAGC CIGGIGGIGG IGGIAGIG 360 300

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

(B) TYPE: (A) LENGTH: Amino acid

(C) TOPOLOGY:

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

Val Cys His Val Ile Phe Lys Asn Gln Arg Met His Ser Ala Thr Ser 1 10 15

Leu Phe Ile Val Asn Leu Ala Val Ala Asp Ile Met Ile Thr Leu Ile  $20 \ 25 \ 30$ 

Gly Lys Gly Met Cys His Val Ser Arg Phe Ala Gln Tyr Cys Ser Leu  $50 \\ 60$ Asn Thr Pro Phe Thr Leu Val Arg Phe Val Asn Ser Thr Trp Ile Phe 35 40 45

His Val Ser Ala Leu Thr 65

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS: (A) LENGTH:

Amino acid

(B) TYPE: (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE:

Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

Glu Pro Ala Asp Leu Phe Trp Lys Asn Leu Asp Leu Pro Thr Phe Ile 1 15

Leu Leu Asn Ile Leu Pro Leu Leu Ile Ile Ser Val Ala Tyr Val Arg  $20 \ 25 \ 30$ 

Val Thr Lys Lys Leu Trp Leu Cys Asn Met Ile Val Asp Val Thr Thr 35

Glu Gln Tyr Phe Ala Leu Arg Pro Lys Lys Lys Lys Thr Ile Lys Met 50 55 60

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Leu Met Leu Val Val Leu 65
(2) INFORMATION FOR SEQ ID NO: 36;
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 210 (B) TYPE: Nucleic acid (C) STRANDEDNESS: Double (D) TOPOLOGY: Linear
(ii) MOLECULE TYPE: CDNA
(ix) FEATURE (C) IDENTIFICATION METHOD: S
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36;
GICTGICAIG ICAICTICAA GAACCAGGGA AIGCACICGG CCACCAGCCI CTICAICGIC 60
•
(2) INFORMATION FOR SEQ ID NO: 37;
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 213 (B) TYPE: Nucleic acid (C) STRANDEDNESS: Double (D) TOPOLOSY: Linear
(ii) MOLECULE TYPE: CDNA
(ix) FEATURE (C) IDENTIFICATION METHOD: S
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:
GAGCCAGCTG ACCTCTTCTG GAAGAACCTG GACTTGCCCA CCTTCATCCT GCTCAACATC 60
CTGCCCCTCC TCATCATCT TGTGGCCTAC GTTCGTGTGA CCAAGAAACT GTGGCTGTGT 120

9 120 180 210

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	38:
	SEQ ID NO: 38
	a
1	SEQ
	DESCRIPTION:
	SEQUENCE
	(xi)

Ala Ser Trp His Lys Arg Gly Gly Arg Arg Ala Ala Trp Val Val Cys 1 5 10 15

Gly Val Val Trp Leu Ala Val Thr Ala Gln Cys Leu Pro Thr Ala Val 20 30

Phe Ala Ala Thr Gly Ile Gln Arg Asn Arg Thr Val Cys Tyr Asp Leu 35

Ser Pro Pro Ile Leu Ser Thr Arg Tyr Leu Pro Tyr Gly Met Ala Leu 50 60

Thr Val Ile Gly Phe Leu Leu Pro Phe Ile Ala Leu Leu Ala Cys Tyr 65 70 75 80

Cys Arg Met Ala Arg Arg Leu Cys Arg Gln Asp Gly Pro Ala Gly Pro 85 90 Val Ala Gin Giu Arg Arg Ser Lys Ala Ala Arg Met Ala Val Val 100 100

Ala Ala Val 115

## (2) INFORMATION FOR SEQ ID NO: 39:

Amino acid (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 328
(B) TYPE: Amino ac
(C) TOPOLOGY: Linear

Linear

### Peptide (ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

Met Glu Gln Asp Asn Gly Thr Ile Gln Ala Pro Gly Leu Pro Pro Thr  $_{\mbox{\scriptsize 1}}$ 

9

AATATGATTG TCGATGTGAC CACAGAGCAG TACTTTGCCC TGCGGCCCAA AAAGAAGAAG 180

ACCATCAAGA TGTTGATGCT GGTGGTAGTC CTC

(2) INFORMATION FOR SEQ ID NO: 38:

Amino acid

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 115
(B) TYPE: Amino ac:
(C) TOPOLOGY: Linear

213

Thr Cys Val Tyr Arg Glu Asp Phe Lys Arg Leu Leu Leu Thr Pro Val 25 30

Tyr Ser Val Val Leu Val Val Gly Leu Pro Leu Asn Ile Cys Val Ile 35 46

Ala Gln Ile Cys Ala Ser Arg Arg Thr Leu Thr Arg Ser Ala Val Tyr 50 60

Thr Leu Asn Leu Ala Leu Ala Asp Leu Met Tyr Ala Cys Ser Leu Pro 65 75 80

.

Leu Leu Ile Tyr Asn Tyr Ala Arg Gly Asp His Trp Pro Phe Gly Asp 95 Leu Ala Cys Arg Phe Val Arg Phe Leu Phe Tyr Ala Asn Leu His Gly 100

Ser Ile Leu Phe Leu Thr Cys Ile Ser Phe Gln Arg Tyr Leu Gly Ile 115 120 125

Cys His Pro Leu Ala Ser Trp His Lys Arg Gly Gly Arg Arg Ala Ala 135 136 Trp His Lys Arg Gly Gly Arg Arg Ala Ala 137 Try No. 1 Try Ala Gln Cys Leu

Trp Val Val Cys Gly Val Val Trp Leu Ala Val Thr Ala Gln Cys Leu 145 150 150 155 160 Pro Thr Ala Val Phe Ala Ala Thr Gly Ile Gln Arg Asn Arg Thr Val 175 170 175

Cys Tyr Asp Leu Ser Pro Pro Ile Leu Ser Thr Arg Tyr Leu Pro Tyr 180 - 185 - 180 - 185 - 190 Pro Phe Ile Ala Leu

Gly Met Ala Leu Thr Val Ile Gly Phe Leu Leu Pro Phe Ile Ala Leu 195 Leu Ala Cys Tyr Cys Arg Met Ala Arg Arg Leu Cys Arg Gln Asp Gly 210 210

Pro Ala Gly Pro Val Ala Gln Glu Arg Arg Ser Lys Ala Ala Arg Met
240
225

Ala Val Val Ala Ala Val Phe Ala Ile Ser Phe Leu Pro Phe His 255 250

Ile Thr Lys Thr Ala Tyr Leu Ala Val Arg Ser Thr Pro Gly Val Ser 260 265 270 287 Thr Phe Ala Ala Ala Tyr Lys Gly Thr Arg Pro

Cys Pro Val Leu Glu Thr Phe Ala Ala Tyr Lys Gly Thr Arg Pro 275 280

Phe Ala Ser Val Asn Ser Val Leu Asp Pro Ile Leu Phe Tyr Phe Thr 290 295

Gln Gln Lys Phe Arg Arg Gln Pro His Asp Leu Leu Gln Arg Leu Thr 305 310

Ala Lys Trp Gln Arg Gln Arg Val 325

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 345
(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double (D) TOPOLOGY: Linear

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(ii) MOLECULE TYPE: CDNA

(ix) FEATURE (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

GCTTCCTGGC ACAAGCGTGG AGGTCGCCGT GCTGCTTGGG TAGTGTGTGG AGTCGTGTGG CTGGCTGTGA CAGCCCAGTG CCTGCCCACG GCAGTCTTTG CTGCCACAGG CATCCAGCGC 120
AACCGCACTG TGTGCTACGA CCTGAGGCCCA CCCATCCTGT CTACTCGCTA CCTGCCCTAT 180
GGTATGGCCC TCACGGTCAT CGGCTTCTTG CTGCCCTTCA TAGCCTTACT GGCTTGTTAT 240
TGTCGCATGG CCCGCCGCT GTGTCGCCAG GATGGCCCAG CAGGTCCTGT GGCCCAAGAG 300
CCGGCGCAGCA AGGCGGCTCG TATGGCTGTG GTGGTGGCAG CTGTC 345

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

A) LENGTH:

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

CTGCCACTGA ACATCTGCGT CATTGCCCAG ATCTGCGCAT CCCGCCGGAC CCTGACCGGT CCTGAGGATT TCAAGCGACT GCTGCTAACC CCGGTATACT CGGTGGTGCT GGTGGTCGGC ATGGAGCAGG ACAATGGCAC CATCCAGGCT CCAGGCTTGC CGCCCACCAC CTGCGTCTAC TITGTACGCT TCCTCTTCTA TGCCAATCTA CATGGCAGCA TCCTGTTCCT CACCTGCATT CTACTTATCT ATAACTACGC CAGAGGGGAC CACTGGCCCT TCGGAGACCT CGCCTGCCGC TOOGCTGTGT ACACCCTGAA CCTGGCACTG GCGGACCTGA TGTATGCCTG TTCACTACCC COCCETECTS CTTGGGTAGT GTGTGGAGTC GTGTGGCTGG CTGTGACAGC CCAGTGCCTG AGCTTCCAGC GCTACCTGGG CATCTGCCAC CCCCTGGCTT CCTGGCACAA GCGTGGAGGT CCCACGGCAG TCTTTGCTGC CACAGGCATC CAGCGCAACC GCACTGTGTG CTACGACCTG TTCTTGCTGC CCTTCATAGC CTTACTGGCT TGTTATTGTC GCATGGCCCG CCGCCTGTGT AGCCCACCCA TCCTGTCTAC TCGCTACCTG CCCTATGGTA TGGCCCTCAC GGTCATCGGC 180 660 540 420 300 600 480

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TG 720	CA 780	CT 840				•														
: GGCTCGTATG	CACCAAGACA	GACCTTCGCT	CCCCATICIC	GAGGCTCACA						Arg Arg	Gly Phe	His Gln	Gln Trp	Val Phe 80	Lys Val 95	Ser Glu	Val Val			
GCAGCAAGGC	CTTTCCACAT	CTGTGCTGGA	GTGTTCTGGA	ATCTCTTACA					42:	Val His Ser	Leu Gly Val	Val Ala Tyr 45	Cys Trp Glu 60	Cys Thr Phe	Tyr Ala	Lys Lys 110	Val Val 125			
CAAGAGCGGC	AGCTTCCTGC CTTTCCACAT	GPCTCTTGCC	AGTGTCAACA	CAACCCCACG		-	i: acid		ID NO:	Ala Ile 10	Ala Leu	Ser Pro	Thr Phe	Val Val Cy 75	Cys Phe Cys 90	Asn Met Ser	Val Leu Val		acid	
TCCTGTGGCC (	CTTTGCCAIC A	CACGCCCGGT G	GCCCTTCGCC A	orreceeses e	J.	ID NO: 42:	CHARACTERISTICS H: 128 Amino a	Peptide	DESCRIPTION: SEQ	Arg Tyr Val	Ser Arg Asn 25	Ala Met Ala 40	Ser Asn Gln 55	Lys Ala Tyr	Leu Ile	s Leu Lys 105	Gln Thr 120	NO: 43:	RISTICS: 384 Nucleic Double Linear	CDNA
	TGGCAGCTGT CT	CTGTGCGCTC CA	AAGGCACTCG GC	CACAACAGAA GT	AGAGGCAGAG AGTC	N FOR SEQ ID	SEQUENCE CHARA(A) LENGTH: (B) TYPE: (C) TOPOLOGY:	CULE TYPE:		Val Asp 5	Arg Val	Ser Ile	Arg Asp	His Lys 70	Pro Leu Leu 85	His Lys Lys	Lys Thr Ala	FOR SEQ ID		E TYPE:
				TTČTACTTCA CAC	GCCAAGTGGC AGA	INFORMATION FOR	(i) SEQU (A) (B) (B) (C) (C)	(ii) MOLECULE	(xi) SEQUENCE	Ala Met Ser	er Ser Leu 20	Trp Ala Leu 35	eu Phe His 50	n Lys Leu	r Leu Leu	n His Leu 100	Lys Lys 115	INFORMATION	SEQU (A) (B) (C) (D)	(ii) MOLECULE
၁၁၅၁	90 <u>7</u> 0	600	GCTG	TTĊŢ	GCCA	(2) 1				Ala A.	Ser Se	Ile Tr	Arg Leu 50	Pro Asn 65	біу туг	Leu Asn	Ala Ser	(2) INF	(i)	i)

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	METHOD:
) FEATURE	(C) IDENTIFICATION

43:
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B
SEQ
DESCRIPTION:
SEQUENCE
(xi)

9	3 5			40	300	360
GCCGCGAIGT CIGIGGAICG CTACGIGGCC ATTGIGCACT CGCGGCGCTC CICCICCETC	AGGSTGTCCC GCAACGCACT GCTGGGCGTG GGCTTCATCT GGGCGCTGTC CATCGCCATG	GCCTCGCCGG TGGCCTACCA CCAGCGTCTT TTCCATCGGG ACAGCAACCA GACCTTCTGC	TGGGAGCAGT GGCCCAACAA GCTCCACAAG AAGGCTTACG TGGTGTGCAC TTTTCGTCTTTTTT		TAMICATORS	GACTOCACAG
CGCGGCGCTC	GGCGCTGTC	ACAGCAACCA	TGGTGTGCAC	GGGTACCTTC TGCCTTACT GCTCATCTGC TTTTGCTATG CC12GCTCTT T11001111	CATABABAGC TGABABAGAT GTCABABABG TCTGABGCGT CCABABABA CACAGAAAAAAAAAAAAAAAAAAAAAAAA	von and and and and and and and and and an
ATTGTGCACT	GGCTTCATCT	TTCCATCGGG	AAGGCTTACG	TTTTGCTATG	TCTGAAGCAT	
CTACGTGGCC	GCTGGGCGTG	CCAGCGTCTT	GCTCCACAAG	GCTCATCTGC	GTCAAAAAG	AGTA
CTGTGGATCG	GCAACGCACT	TGGCCTACCA	GGCCCAACAA	TGCCCTTACT	TGAAAAACAT	TGGTCGTTGT
GCCGCGATGT	AGGGTGTCCC	GCCTCGCCGG	TGGGAGCAGT	GGGTACCTTC	CATAAAAGC	ACCGTCCTGG TGGTCGTTGT AGTA

## (2) INFORMATION FOR SEQ ID NO: 44:

- Amino acid Linear (i) SEQUENCE CHARACTERISTICS: (A) LENGTH:
  (B) TYPE:
  (C) TOPOLOGY:
- (ii) MOLECULE TYPE: Peptide

# (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

Val Leu Trp Phe Phe Gly Phe Ser Ile Lys Arg Thr Pro Phe Ser Val  $_{
m 10}$ 

Tyr Phe Leu His Leu Ala Ser Ala Asp Gly Ala Tyr Leu Phe Ser Lys 25 30

Ala Val Phe Ser Leu Leu Asn Ala Gly Gly Phe Leu Gly Thr Phe Ala 40

His Tyr Val Arg Ser Val Ala Arg Val Leu Gly Leu Cys Ala Phe Val 50 60

Ala Gly Val Ser Leu Leu Pro 65

## (2) INFORMATION FOR SEQ ID NO: 45:

- (i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 215
  (B) TYPE: Nucleic acid
  (C) STRANDEDNESS: Double
  (D) TOPOLOGY: Linear
  - Nucleic acid

### (ii) MOLECULE TYPE:

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Ala	Ser 145	Met	Tyr	Ala	Asp	Lys 65	Leu	Asn	Glu	Met 1				(2)	16	୍ଷ	IJ	ន្ន		
Leu	Leu	ser 130	Phe	Leu	Leu	Pro	- G1y 50	Phe	l Pro	; Glu		_	_		255	99	.660	CIO	_	
Ser	Arg	Val	Phe 115	Pro	Ala	Arg	Asn	35	Pro	ı Leu	(xi)	(ii)	(±)	FORM	TGCGCCTTCG	GCCGCTTCC	CTGGCCAGCG	GIGCICIGGI	(xi)	(ix)
Ile	Val	Asp	Thr	100	Tyr	Ser	) Ser	Thr	20 Ala	u Ala	SEQU	MOLE	SEQU (A) (B) (C)	ATIC					SEQ	(C)
Ala 165	Ser	Arg	Va]	Trp	Leu 85	Thr	Leu	. Leu	Pro	Met 5	SEQUENCE	MOLECULE	SEQUENCE CH (A) LENGTH: (B) TYPE: (C) TOPOLOG	INFORMATION FOR	Č G G	TGGGCACCIT	CCGACGGCGC	TCTTCGGCTT	SEQUENCE	FEATURE (C) IDEN
Met	Arg 150	Tyr	Ser	Val	Leu	Thr 70	ı Val	ı Val	nts c	t Val	DES	TYPE:		R SEQ	CGT		CGC			WIIF:
Ala	Àsn	Val 135	Met	Leu	Phe	Asn	11e 55	l Val	ı Ser	l Asn	CRIP		RACT	ğ	GAGO	2292	CTAC	CTCC	CRII	[CAT]
Ser	Ala	Ála	Leu 120	Gly	Cys	Leu	Thr	Phe 40	: Arg	1 Leu	DESCRIPTION:	Pel	CHARACTERISTICS H: 348 Amino Amino Linear	Ö	TEGCEGECET GAGCCTCCTG	CGCCCACTAT	CTACCTCTTC	CTCCATCAAG	DESCRIPTION:	URE IDENTIFICATION METHOD:
Pro	Leu	Ile	Val	Ala 105	Ile	Phe	Val	Gly	) Pro 25	Ser	SEQ	Peptide	ISTICS 348 Amino : Linear	46:						ETHC
Val 170	Leu	Va1	Ser	Phe	Pro 90	Ile	Leu	Leu	Leu	10 : Glu	ğ	w	acid		CCGGC	GTGCGCAGCG	AGCAAGGCCG	AGGACCCCCT	SEQ I	
Ala	Gly 155	His	Ile	Ile	Phe	Leu 75	Ala	Ile	Phe	Gly	NO:					CAGO	9	റാാ	ID NO:	S
Tyr	Val	Ser 140	Phe	Cys	G1n	Asn	Arg	Phe	Gly	Åsn	46:								45:	
His	Gly	Àrg	Thr 125	Lys	Ala	Leu	Ser	Ala 45	Ile	Gly						TGGCCCGGGT	TGTTCTCCCT	TCTCCGTCTA	••	
G1n	Phe	Arg	Leu	Phe 110	Thr	Ser	Lys	Met	G1y 30	Ser						ğ	CCT	TCTA		
Arg :	Ile '	Ser	Ala	Ile	Va1 95	11e	Pro	Gly	Val	Asp 15						eCT.	CTC	CIT		
Leu	Trp 160	Ser	Ala	His	Туг	Ala 80	Gly	Val	Glu	Pro						GCTGGGGCTC	GCTGAACGCC	CTTCCTGCAC		
															219	180	12	<u>ق</u>		

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Phe His Arg Asp Ser Asn Gln Thr Phe Cys Trp Glu Gln Trp Pro Asn 180 185 Lys Leu His Lys Lys Ala Tyr Val Val Cys Thr Phe Val Phe Gly Tyr 195 200 205

His Leu His Lys Lys Leu Lys Asn Met Ser Lys Lys Ser Glu Ala Ser 225 230 235 Leu Leu Pro Leu Leu Ile Cys Phe Cys Tyr Ala Lys Val Leu Asn 215 220

Lys Lys Thr Ala Gln Thr Val Leu Val Val Val Val Val Phe Gly 245 250

Ile Ser Trp Leu Pro His His Val Val His Leu Trp Ala Glu Phe Gly 265 270

Ala Phe Pro Leu Thr Pro Ala Ser Phe Phe Phe Arg Ile Thr Ala His 275 280 285

Leu Ser Glu Asn Phe Arg Lys Ala Tyr Lys Gln Val Phe Lys Cys His 305 310 315

Met Asp Thr Pro Pro Ser Thr Asn Cys Thr His Val Val Cys Asp Glu Ser Pro Arg Ser Glu Thr Lys Glu Asn Lys Ser Arg 325 330 335

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS: (A) LENGTH:

(B) TYPE: Nucleic (C) STRANDEDNESS: Double (D) TOPOLOGY: Linear Nucleic acid

(ii) MOLECULE TYPE: CDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

AGCAAACCAG GCAACCCCCG CAGCACCACC AACCTGTTTA TCCTCAATCT GAGCATCGCA GGCCTGATTT TCGCGATGGG CGTGCTGGGC AACAGCCTGG TGATCACCGT GCTGGCGCGC CCGGAGTCCA GGCCGCTCTT CGGCATTGGC GTGGAGAACT TCATTACGCT GGTAGTGTTT ATGGAACTGG CTATGGTGAA CCTCAGTGAA GGGAATGGGA GCGACCCAGA GCCGCCAGCC 240 180 120 60

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	Ser Arg Phe Ile Leu Gly Phe Leu Leu Pro Phe Leu Val Ile Gly Phe
	Lys Glu Met Gln Thr Val Arg Gln Trp Ile His Ala Thr Cys Phe Ile 65
	Arg Val Thr Cys Arg Asn Asn Tyr Ala Val Ser Thr Asp Trp Glu Ser 50
	Ser Val Pro Tyr Leu Val Phe Arg Glu Thr Tyr Asp Asp Arg Lys Gly 35
	Trp Ala Ser Arg Val Val Leu Gly Val Trp Leu Ser Ala Thr Ala Phe $20\ \ 25$
	Leu Leu Thr Leu His Pro Val Trp Ser Gln Lys His Arg Thr Ser His 1 $^{\rm 15}$
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:
	(ii) MOLECULE TYPE: Peptide
	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 125 (B) TYPE: Amino acid (C) TOPOLOGY: Linear
	CCATCCACCA ACTGCACCCA CGTG (2) INFORMATION FOR SEQ ID NO: 48:
1044	CAGIGARACT ENGINEERS:
1020	ECKKOWOKO CONTOURN
096	
900	
840	CCCCATCATG TCGTCCACCT CTGGGCTGAG ITTGGAGCCT TCCCACTGAC GCCAGCTTCC
780	AAGAAAAAGA CTGCACAGAC CGTCCTGGTG GTCGTTGTAG TATTTGGCAT ATCCTGGCTG
720	AAGGECCTTA ATCAECTGCA TAAAAAGCTG AAAAACATGT CAAAAAAGTC TGAAGCAECC
099	GIGIGGACIT ICGICITIGG GIACCITCIG CCCTIACIGC ICAICIGCIT INGCIAIGCC
909	AGCAACCAGA CCTTCTGCTG GGAGCAGTGG CCCAACAAGC TCCACAAGAA GGCTTACGTG
540	GCGCTGTCCA TCGCCATGGC CTCGCCGGTG GCCTACCACC AGCGTCTTTT CCATCGGGAC
480	CGGCGCTCCT CCTCCTCAG GGTGTCCCGC AACGCACTGC TGGGCGTGGG CTTCATCTGG
420	GTGAGCATCT TCACCCTGGC CGCGATGTCT GTGGATCGCT ACGTGGCCAT TGTGCACTCG
360	TGGGTGCTGG GCGCCTTCAT CTGCAAGTTT ATACACTACT TCTTCACCGT GTCCATGCTG
300	GACCTGGCCT ACCTGCTCT CTGCATCCCT TTTCAGGCCA CCGTGTATGC ACTGCCCACC

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Lys
Phe
Leu 110
Gly
Arg
Glu
Lys
Met 105
Lys
Arg
Ala
Val
Arg 100
Glu
Tyr
Cys

Ser Ser Lys Pro Phe Lys Val Thr Met Thr Ala Val Ile 115

- (2) INFORMATION FOR SEQ ID NO: 49:
- (i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 377
  (B) TYPE: Nucleic ac
  (C) STRANDEDNESS: Double
  (D) TOPOLOGY: Linear
- Nucleic acid
- CDNA (ii) MOLECULE TYPE:
- (ix) FEATURE
- (C) IDENTIFICATION METHOD: S
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

GTAGCCCGCA AGATGAAAGA GAGGGCCCTC TTTAAATCCA GCAAACCCTT CAAAGTCACG GACTEGGAAA GEAAAGAGAT GEAAACAGTA AGACAATGGA ITCATGCCAC CTGTTTCATC AGCCGCTTCA TACTGGGCTT CCTTCTGCCT TTCTTAGTCA TTGGCTTTTG TTATGAAAGA CTICTCACCC TICACCCAGI GIGGICCCAA AAGCACCGAA CCICACACIG GGCTICCAGA GICGITCTGG GAGICTGGCT CICTGCCACT GCCITCAGCG IGCCCTAITT GGITTICAGG GAGACATATG ATGACCGTAA AGGAAGAGTG ACCTGCAGAA ATAACTACGC TGTGTCCACT ATGACTGCTG TTATCTC

180 240 300 360 377

- (2) INFORMATION FOR SEQ ID NO: 50:
- Amino acid (i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 119
  (B) TYPE: Amino ac.
  (C) TOPOLOGY: Linear
- Peptide (ii) MOLECULE TYPE:
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

Phe Lys Ile Val Lys Pro Leu Ser Thr Ser Phe Ile Gln Ser Val Asn  $_{1}^{\rm A}$  .

Tyr Ser Lys Leu Val Ser Leu Val Val Trp Leu Leu Met Leu Leu Leu 25  $20\,$ 

Ala Val Pro Asn Val Ile Leu Thr Asn Gln Arg Val Lys Asp Val Thr 35

Gln Ile Lys Cys Met Glu Leu Lys Asn Glu Leu Gly Arg Gln Trp His 50  $\,$  60

Lys Ala Ser Asn Tyr Ile Phe Val Gly Ile Phe Trp Leu Val Phe Leu 65 70 75 80

His Leu Lys Ser Arg Lys Asn Ser Ile Ser Val Lys Lys Ser Ser 100 105 110

Leu Leu Ile Ile Phe Tyr Thr Ala Ile Thr Arg Lys Ile Phe Lys Ser 90 95

Arg Asn Ile Phe Ser Ile Val

(2) INFORMATION FOR SEQ ID NO: 51:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH:
- (B) TYPE: Nucleic (C) STRANDEDNESS: Double (D) TOPOLOGY: Linear Nucleic acid
- (ii) MOLECULE TYPE: CDNA
- (ix) FEATURE
- (C) IDENTIFICATION METHOD: S
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:
- AACCAGAGAG TTAAGGACGT GACGCAGATA AAATGCATGG AACTTAAAAA CGAACTGGGC GTCTCGCTGG TGGTCTGGTT GCTCATGCTC CTCCTCGCCG TCCCCAACGT CATTCTCACC TICAAGATIG IGAAGCCCCI TICCACGICC TICAICCAGI CIGIGAACIA CAGCAAACIC 120
- CGCCAGTGGC ACAAGGCGTC AAACTACATC TTTGTGGGCA TTTTCTGGCT TGTGTTCCTT
- TIGCTAATCA TITICTACAC TGCTATCACC AGGAAAATCT TTAAGTCCCA CCTGAAATCC 300
- AGAAAGAATT CCATCTCGGT CAAAAAGAAA TCTAGCCGCA ACATCTTCAG CATCGTG
- (2) INFORMATION FOR SEQ ID NO: 52:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH:
- (B) TYPE: (C) TOPOLOGY: Linear Amino acid
- (ii) MOLECULE TYPE: Peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

Val Asp Leu Leu Ala Ala Leu Thr Leu Met Pro Leu Ala Met Leu Ser 1 10 15

Leu Tyr Leu Phe Leu Ser Val Cys Phe Val Ser Leu Ala Ile Leu Ser  $35 \hspace{1cm} 40 \hspace{1cm} 45$ Ser Ser Ala Leu Phe Asp His Ala Leu Phe Gly Glu Val Ala Cys Arg
20 25 30

Val Ser Ala Ile Asn Val Glu Arg Tyr Tyr Tyr Val Val His Pro Met 50 55 60

Gly Val Trp Val Lys Ala Leu Ala Met Ala Ser Val Pro Val Leu Gly 85 90 95 Arg Tyr Glu Val Arg Met Lys Leu Gly Leu Val Ala Ser Val Leu Val  $65\,$   $70\,$   $75\,$   $80\,$ 

Arg Val Ser Trp Glu Glu Gly Pro Pro Ser Val Pro Pro Gly Cys Ser 100 105

Leu Gln Trp Ser His Ser Ala Tyr Cys Gln Leu Phe Val Val Val Phe 115 120 125

Ala Val Leu Tyr Phe Leu Leu Pro Leu Leu Leu Ile Leu Val Val Tyr 130 135 140

Cys Ser Met Phe Arg Val Ala Arg Val Ala Ala Met Gln His Gly Pro 145 150 155

Leu Pro Thr Trp Met Glu Thr Pro Arg Gln Arg Ser Glu Ser Leu Ser 170 175

Ser Arg Ser Thr Met Val Thr Ser Ser Gly Ala Pro Gln Thr Thr Pro 180 185

His Arg Thr Phe Gly Gly Gly Lys Ala Ala Val Val Leu Leu Ala Val 195 200 205

Gly Gly Gln Phe Leu Leu Cys Trp Leu Pro Tyr Phe Ser Phe His Leu 210 215 220

Tyr Val Ala Leu Ser Ala Gln Pro Ile Ala Ala Gly Gln Val Glu Asn 225 230 235

Val Val Thr Trp Ile Gly Tyr Phe Cys Phe Thr Ser 245 (2) INFORMATION FOR SEQ ID NO: 53:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: Nucleic acid
- (B) TYPE: Nucleic (C) STRANDEDNESS: Double (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE:

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	METHOD:
FEATURE	(C) IDENTIFICATION
(xt)	

756			ACCTCC	GREGREACT GGATTGGCTA CTTCTGCTTC ACCTCC	GGATTGGCTA	GTGGTGACCT
720	GGTGGAGAAC	CAGCGGGGCA	CAGCCCATTG	TCTTCCACC TCTAIGTGGC CCTGAGCGCT CAGCCCATTG CAGCGGGGCA GGTGGAGAAC	TCTATGTGGC	TCCTTCCACC
099	GCCCTACTTC	TCTGTTGGTT	CAGTTCCTGC	GCAGCAGTGG TCCTCCTGGC TGTGGGAGGA CAGTTCCTGC TCTGTTGGTT GCCCTACTTC	recreases	GCAGCAGTGG
009	CGGAGGGAAG	GGACGTTTGG	ACCCCTCACC	ATGGTCACCA GCTCGGGGGC CCCGCAGACC ACCCCTCACC GGACGTTTGG CGGAGGGAAG	GCTCGGGGG	ATGGTCACCA
540	CCGCTCCACT	CTCTCAGCAG	CGCTCCGAGT	CTGCCCACGT GGATGGAGAC GCCCCGGCAA CGCTCCGAGT CTCTCAGCAG CCGCTCCACT	GGATGGAGAC	CTGCCCACGT
480	GCACGGGCCG	CTGCCATGCA	GCTCGTGTGG	CTISTGSTCT ACTGCAGCAT GTTCCGGGTG GCTCGTGTGG CTGCCATGCA GCACGGGCCG	ACTGCAGCAT	CTTGTGGTCT
420	GCTCCTCATC	recreceer	CTCTACTTCC	IGCCAGCITI ICGIGGIGGI CIICGCCGIC CICIACTICC IGCIGCCCCI GCICCICAIC	rcerecreer	TGCCAGCTTT
360	CAGTGCCTAC	AATGGAGCCA	TGTTCACTCC	GAGGAAGGCC CTCCCAGTGI CCCCCCAGGC TGITCACTCC AAIGGAGCCA CAGIGCCIAC	CTCCCAGTGT	GAGGAAGGCC
300	GGTGTCCTGG	TGTTGGGAAG	TCTGTGCCAG	GECCIGIGGE TGAAGGCCCI GGCCAIGGCI TCTGTGCCAG IGTIGGGAAG GGTGTCCTGG	TGAAGGCCCT	GCCGTGTGGG
240	TGTGCTGGTG	тестесстс	AAACTGGGGC	GTCCACCCCA TGCGCTATGA GGTGCGCATG AAACTGGGGC TGGTGGCCTC TGTGCTGGTG	TGCGCTATGA	GTCCACCCCA
180	CTATTATGTG	TGGAGCGCTA	GCCATCAATG	TITICICAGCC IGGCCAICCI CICGGIGICC GCCAICAAIG IGGAGCGCTA CIAITAIGIG	TGGCCATCCT	TTTGTCAGCC
120	GAGCGTCTGC	ACTIGITCCT	тессесстст	TITGACCACG CCCICITIGG GGAGGIGGCC IGCCGCCICT ACTIGITCCI GAGCGICTGC	CCCTCTTTGG	TTTGACCACG
09	CICCGCCCIC	TGCTCTCCAG	CCTCTGGCCA	GIGGACCIGC IGGCIGCCCI GACCCICAIG CCICIGGCCA IGCICICCAG CICCGCCCIC	TGGCTGCCCT	GTGGACCTGC
		53:	SEQ ID NO:	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:	SEQUENCE DE	(x)

# (2) INFORMATION FOR SEQ ID NO: 54:

- SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 263
  (B) TYPE: Amino ac
  (C) TOPOLOGY: Linear <u>:</u>
- Amino acid Linear
- Peptide (ii) MOLECULE TYPE:

# (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

Lys Ser Thr Ala Arg Ala Arg Gly Ser Ile Leu Gly Ile Trp Ala 65 75 80 Ala Asp Val Leu Val Thr Ala Ile Cys Leu Pro Ala Ser Leu Leu Val 1 10 15 Asp lle Thr Glu Ser Trp Leu Phe Gly His Ala Leu Cys Lys Val Ile 20 25 Pro Tyr Leu Gln Ala Val Ser Val Val Val Leu Thr Leu Ser 35 Ser Ile Ala Leu Asp Arg Trp Tyr Ala Ile Cys His Pro Leu Leu Phe 50 60

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Ser Asp Arg Glu Ala Ile Tyr Ala Cys Phe Thr Phe Ser His Trp Leu Ser Val Leu Asn Val Leu Lys Arg Val Phe Gly Met Phe Arg Gln Ala 225 Phe Phe Ile Val Thr Tyr Leu Ala Pro Leu Gly Leu Met Ala Met Ala Thr Ser Ala Leu Val Arg Asn Trp Lys Arg Pro Ser Asp Gln Leu Asp Asp Gln Gly Gln Gly Leu Ser Glu Pro Gln Pro Arg Ala Arg Ala 180 180 Met Leu Met Val Val Leu Leu Val Phe Ala Leu Cys Tyr Leu Pro Ile  $210\,$ Val Ser Leu Ala Val Met Val Pro Gin Ala Ala Val Met Glu Cys Ser Ser Val Leu Pro Glu Leu Ala Asn Arg Thr Arg Leu Leu Ser Val Cys Asp Glu Arg Trp Ala Asp Asp Leu Tyr Pro Lys Ile Tyr His Ser Cys Tyr Phe Gln lle Phe Arg Lys Leu Trp Gly Arg Gln lle Pro Gly Thr 145 Phe Leu Ala Glu Val Lys Gln Met Arg Ala Arg Arg Lys Thr Ala Lys

Val Tyr Ala Asn Ser Ala Ala 260

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:

Nucleic acid (A) LENGTH: 789 (B) TYPE: Nucleic (C) STRANDEDNESS: Double (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE:

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

120 9 TCTGGCTCT INGGCCATGC CCTCTGCAAG GTCATCCCCT ATCTACAGGC CGTGTCCGTG GCCGATGTGC TGGTGACAGC CATCTGCCTG CCGGCCAGTC TGCTGGTAGA CATCACGGAA

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ACCTCGGCCC TGGTGCGCAA CTGGAAGCGG CCCTCAGACC AGCTGGACGA CCAGGGCCAG GGCCTGAGCT CAGAGCCCCA GCCCCGGGCC CGCGCCTTCC TGGCCGAGGT GAAACAGATG ATGGCCATGG CCTATTTCCA GATCTTCCGC AAGCTCTGGG GCCGCCAGAT CCCCGGCACC TACCCCAAGA TCTACCACAG CTGCTTCTTC ATTGTCACCT ACCTGGCCCC ACTGGGCCTC GAGCTGGCCA ACCGCACCCG CCTCCTGTCT GTCTGTGATG AGCGCTGGGC AGACGACCTG GTGTCGCTGG CTGTCATGGT GCCTCAGGCT GCTGTCATGG AGTGTAGCAG CGTGCTGCCC CCGCTGTTGT TCAAGAGCAC TGCCCGGCGC GCCCGCGGCT CCATCCTCGG CATCTGGGCG TACCTGCCCA TCAGTGTCCT CAACGTCCTC AAGAGGGTCT TCGGGATGTT CCGCCAAGCC CGAGCCCGGA GGAAGACGGC CAAGATGCTG ATGGTGGTGC TGCTGGTCTT CGCCCTCTGC TCAGTGGTCG TGCTGACTCT CAGCTCCATC GCCCTGGACC GCTGGTACGC CATCTGCCAC AGCGACCGAG AGGCCATCTA CGCCTGCTTC ACCTTCTCCC ACTGGCTGGT GTACGCCAAC 780 600 540 360 300 720 660 480 420

### (2) INFORMATION FOR SEQ ID NO: 56:

- (i) SEQUENCE CHARACTERISTICS: (A) LENGTH:
- (B) TYPE: Linear Amino acid
- (ii) MOLECULE TYPE: Peptide

# (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

Thr Cys Val Tyr Arg Glu Asn Phe Lys Gln Leu Leu Leu Pro Pro Val 20 25 30 Met Glu Trp Asp Asn Gly Thr Gly Gln Ala Leu Gly Leu Pro Pro Thr 1 5 10

Tyr Ser Ala Val Leu Ala Ala Gly Leu Pro Leu Asn Ile Cys Val Ile 35 40 45 Thr Gln Ile Cys Thr Ser Arg Arg Ala Leu Thr Arg Thr Ala Val Tyr 50 55 60

Thr Leu Asn Leu Ala Leu Ala Asp Leu Leu Tyr Ala Cys Ser Leu Pro 65 70 75 80

Phe Ala Cys Arg Leu Val Arg Phe Leu Phe Tyr Ala Asn Leu His Gly 100 105 110 Leu Leu Ile Tyr Asn Tyr Ala Gln Gly Asp His Trp Pro Phe Gly Asp 85 , 90 95

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Cys His Pro Leu Ala Pro Trp His Lys Arg Gly Gly Arg Arg Ala Ala 130 135 140 Trp Leu Val Cys Val Thr Val Trp Leu Ala Val Thr Thr Gln Cys Leu 145 150 150 Ser Ile Leu Phe Leu Thr Cys Ile Ser Phe Gln Arg Tyr Leu Gly Ile 115 120 125

Cys Tyr Asp Leu Ser Pro Pro Ala Leu Ala Thr His Tyr Met Pro Tyr 180 185 190

Pro Thr Ala Ile Phe Ala Ala Thr Gly Ile Gln Arg Asn Arg Thr Val 175

Gly Met Ala Leu Thr Val Ile Gly Phe Leu Leu Pro Phe Ala Ala Leu 195 200 205

Leu Ala Cys Tyr Cys Leu Leu Ala Cys Arg Leu Cys Arg Gln Asp Gly 210 215 220

Ala Val Val Ala Ala Ala Phe Ala Ile Ser Phe Leu Pro Phe His 245 250 250 Pro Ala Glu Pro Val Ala Gin Glu Arg Arg Gly Lys Ala Ala Arg Met 225 230 230 235

Ile Thr Lys Thr Ala Tyr Leu Ala Val Gly Ser Thr Pro Gly Val Pro 260 265 270

Cys Thr Val Leu Glu Ala Phe Ala Ala Ala Tyr Lys Gly Thr Arg Pro  $275 \ 280 \ \cdot \ 285$ 

Gln Lys Lys Phe Arg Arg Arg Pro His Glu Leu Leu Gln Lys Leu Thr 305 310 315 Phe Ala Ser Ala Asn Ser Val Leu Asp Rro Ile Leu Phe Tyr Phe Thr 290 300

Ala Lys Trp Gln Arg Gln Gly Arg 325

(2) INFORMATION FOR SEQ ID NO: 57:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 984
  (B) TYPE: Nucleic
  (C) STRANDEDNESS: Double
  (D) TOPOLOGY: Linear Nucleic acid
- (ii) MOLECULE TYPE:

(ix) FEATURE
(C) IDENTIFICATION METHOD:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

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984				TCGC	AGAGGCAGGG	SCCARATIGGC AGAGGCAGGG TCGC
096	IIIII CUCHAMMAM GIICCGCCGC CGACCATG AGCTCCTACA GAAACTCACA	AGCTCC	CGACCACATG	GLICCOCCGG	רררשפששפשש	9,11,01,1
006	GUGGUCTACA AAGGLACGCG GCCGTTTGCC AGTGCCAACA GCGTGCTGGA CCCCATCCTC	GCGTGC	AGTGCCAACA	GCCGTTTGCC	AAGGCACGCG	GCGCCCTACA
840	GCCTACCTGG CAGTGGGCTC GACGCCGGGC GTCCCTGCA CTGTATTGGA GGCCTTTGCA	CTGTAI	GTCCCCTGCA	GACGCCGGGC	CAGTGGGCTC	GCCTACCTGG
780	GCCGIGGIGG IGGCIGCIGC CITICCCAIC AGCTICCIGC CITITCACAI CACCAAGACA	CTTT	AGCTTCCTGC	CTTTGCCATC	TGGCTGCTGC	SCCGTGGTGG
720	CGCCAGGATG GCCCGGCAGA GCCTGTGGCC CAGGAGCGGC GTGGCAAGGC GGCCCGCATG	GTGGC4	CAGGAGCGGC	GCCTGTGGCC	GCCCGGCAGA	CGCCAGGATG
099	TICTIGCIGC CCTITIGCIGC CCTGCIGGCC IGCIACIGIC ICCIGGCCTG CCGCCTGTGC	тсста	TGCTACTGTC	cercerece	CCTTTGCTGC	Trecrectee
009	AGCCCGCCTG CCCTGGCCAC CCACTATATG CCCTATGGCA TGGCTCTCAC TGTCATCGGC	TGGCT	CCCTATGGCA	CCACTATATG	CCCTGGCCAC	AGCCCGCCTG
540	STCTG CTATGACCTC	GCACT	CAGCGTAACC	CCCACAGCCA TCTTCGCTGC CACAGGCATC CAGCGTAACC GCACTGTCTG	TCTTCGCTGC	CCCACAGCCA
480	CGCCGGGCTG CCTGGCTAGT GTGTGTAACC GTGTGGCTGG CCGTGACAAC CCAGTGCCTG	CCGTG	GTGTGGCTGG	GTGTGTAACC	CCTGGCTAGT	CGCCGGGCTG
420	AGCITCCAGC GCTACCTGGG CATCTGCCAC CCGCTGGCCC CCTGGCACAA ACGTGGGGGC	CCTGG	CCGCTGGCCC	CATCTGCCAC	GCTACCTGGG	AGCTTCCAGC
360	CIGGICCGCI ICCICITCIA IGCCAACCIG CACGGCAGCA ICCICITCCI CACCIGCAIC	TCCTC	CACGGCAGCA	TGCCAACCTG	TCCTCTTCTA	CTGGTCCGCT
300	CTGCTCATCT ACAACTATGC CCAAGGTGAT CACTGGCCCT TTGGCGACTT CGCCTGCCGC	TTGGO	CACTGGCCCT	CCAAGGTGAT	ACAACTATGC	CTGCTCATCT
240	ACGGCCGIGI ACACCCIAAA CCTIGCICIG GCIGACCIGC TAIAIGCCIG CICCCIGCC	TATAT	GCTGACCTGC	ccrrccrcrc	ACACCCTAAA	ACGCCCGTGT
180	CTGCCGCTGA ACATCTGTGT CATTACCCAG ATCTGCACGT CCCGCCGGGC CCTGACCCGC	י מכמפכ	ATCTGCACGI	CATTACCCAG	ACATCTGTGT	CTGCCGCTGA
120	CGCGAGAACT TCAAGCAACT GCTGCTGCCA CCTGTGTATT CGGCGGTGCT GGCGGCTGGC	נ מפמפ	CCTGTGTATT	GCTGCTGCCA	TCAAGCAACT	CGCGAGAACT
09	AISSAAISSS ACAATGSCAC AGGCCAGGCT CTGGGCTTGC CACCCACCAC CTGTGTCTAC	CACCC	CTGGGCTTG	AGGCCAGGCT	ACAATGGCAC	ATGGAATGGG

(2) INFORMATION FOR SEQ ID NO: 58:

SEQUENCE CHARACTERISTICS:

Nucleic acid

(A) LENGTH: 26
(B) TYPE: Nucleic
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

Other nucleic acid Synthetic DNA (ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58;

ACAGCCATCT TCGCTGCCAC AGGCAT

(2) INFORMATION FOR SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS;
(A) LENGTH: 29
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single

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Linear (D) TOPOLOGY:

Other nucleic acid Synthetic DNA (ii) MOLECULE TYPE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

AGACAGTAGC AGGCCAGCAG GGCAGCAAA

(2) INFORMATION FOR SEQ ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:

Nucleic acid (A) LENGTH: 27
(B) TYPE: Nucleic (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear

Other nucleic acid Synthetic DNA (ii) MOLECULE TYPE:

N is inosine (iii) FEATURES: (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

27 CTGTGYGYSA TYGCNNTKGA YMGSTAC

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

Other nucleic acid Synthetic DNA (ii) MOLECULE TYPE:

N is inosine (iii) FEATURES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

AKGWAGWAGG GCAGCCAGCA GANSRYGAA

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- of SEQ ID NO: 1 to SEQ ID NO: 19. represented by a SEQ ID NO selected from the group consisting A DNA which comprises a nucleotide sequence
- protein coupled receptor protein by polymerase chain reaction techniques, which comprises: A method for amplifying a DNA coding for a G
- of a mixture of carrying out a polymerase chain reaction in the presence
- a DNA coding for a G protein coupled receptor protein, said DNA being capable of acting as a template,

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SEQ ID NO: 7, DNA primers comprising a nucleotide primers comprising a nucleotide sequence represented by nucleotide sequence represented by SEQ ID NO: 6, DNA at least one DNA primer selected from the group represented by SEQ ID NO: 5, DNA primers comprising a NO: 3, DNA primers comprising a nucleotide sequence comprising a nucleotide sequence represented by SEQ ID sequence represented by SEQ ID NO: 1, DNA primers consisting of DNA primers comprising a nucleotide

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primers comprising a nucleotide sequence represented by nucleotide sequence represented by SEQ ID NO: 9, DNA represented by SEQ ID NO: 8, DNA primers comprising a NO: 4, DNA primers comprising a nucleotide sequence comprising a nucleotide sequence represented by SEQ ID consisting of DNA primers comprising a nucleotide at least one DNA primer selected from the group SEQ ID NO: 11, DNA primers comprising a nucleotide a nucleotide sequence represented by SEQ ID NO: 18, and sequence represented by SEQ ID NO: 2, DNA primers represented by SEQ ID NO: 16 and DNA primers comprising NO: 14, DNA primers comprising a nucleotide sequence

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comprising a nucleotide sequence represented by SEQ ID sequence represented by SEQ ID NO: 10, DNA primers

> NO: 17 and DNA primers comprising a nucleotide sequence comprising a nucleotide sequence represented by SEQ ID represented by SEQ ID NO: 19; or sequence represented by SEQ ID NO: 15, DNA primers

of a mixture of carrying out a polymerase chain reaction in the presence

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- a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
- NO: 12, and comprising a nucleotide sequence represented by SEQ ID sequence represented by SEQ ID NO: 1 and DNA primers consisting of DNA primers comprising a nucleotide at least one DNA primer selected from the group

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at least one DNA primer selected from the group sequence represented by SEQ ID NO: 13. consisting of DNA primers comprising a nucleotide

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- comprises: a DNA coding for a G protein coupled receptor protein, which A method for screening a DNA library for
- (i) carrying out a polymerase chain reaction in the presence of a mixture of
- said DNA library,

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at least one DNA primer selected from the group a nucleotide sequence represented by SEQ ID NO: 18, and represented by SEQ ID NO: 16 and DNA primers comprising NO: 14, DNA primers comprising a nucleotide sequence comprising a nucleotide sequence represented by SEQ ID sequence represented by SEQ ID NO: 10, DNA primers SEQ ID NO: 7, DNA primers comprising a nucleotide primers comprising a nucleotide sequence represented by nucleotide sequence represented by SEQ ID NO: 6, DNA represented by SEQ ID NO: 5, DNA primers comprising a NO: 3, DNA primers comprising a nucleotide sequence sequence represented by SEQ ID NO: 1, DNA primers at least one DNA primer selected from the group comprising a nucleotide sequence represented by SEQ ID consisting of DNA primers comprising a nucleotide

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primers comprising a nucleotide sequence represented by comprising a nucleotide sequence represented by SEQ ID represented by SEQ ID NO: 8, DNA primers comprising a SEQ ID NO: 17 and DNA primers comprising a nucleotide nucleotide sequence represented by SEQ ID NO: 9, DNA NO: 4, DNA primers comprising a nucleotide sequence SEQ ID NO: 11, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 15, DNA primers sequence represented by SEQ ID NO: 2, DNA primers consisting of DNA primers comprising a nucleotide comprising a nucleotide sequence represented by sequence represented by SEQ ID NO: 19,

under conditions to amplify selectively a template DNA coding for the G protein coupled receptor protein, contained in the DNA library and selecting said DNA; or

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carrying out a polymerase chain reaction in the presence 15

said DNA library

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- comprising a nucleotide sequence represented by SEQ ID sequence represented by SEQ ID NO: 1 and DNA primers consisting of DNA primers comprising a nucleotide at least one DNA primer selected from the group NO: 12, and
  - consisting of DNA primers comprising a nucleotide at least one DNA primer selected from the group seguence represented by SEQ ID NO: 13, 0

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under conditions to amplify selectively a DNA coding for the G protein coupled receptor protein, contained in the DNA library and selecting said DNA.

protein or a fragment thereof, which is obtained by the method A DNA coding for a G protein coupled receptor according to claim 2 to 3.

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5. A G protein coupled receptor protein encoded by the DNA according to claim 4, a peptide segment or fragment thereof or a salt thereof.

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6. A G protein coupled receptor protein comprising an amino acid seguence selected from the group consisting of

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an amino acid sequence represented by SEQ ID NO: 24, an amino seguence represented by SEQ ID NO: 26, an amino acid seguence acid sequence represented by SEQ ID NO: 25, an amino acid represented by SEQ ID NO: 35, an amino acid sequence represented by SEQ ID NO: 38, an amino acid sequence represented by SEQ ID NO: 27, an amino acid sequence represented by SEQ ID NO: 28, an amino acid sequence represented by SEQ ID NO: 34, an amino acid sequence

NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 56; a peptide segment (or fragment) thereof, a modified peptide 34, SEQ ID NO: 35, SEQ ID NO: 38, SEQ ID NO: 39, or SEQ ID NO: the amino acid sequence represented by SEQ ID NO: 24, SEQ ID represented by SEQ ID NO: 56, and substantial equivalents to represented by SEQ ID NO: 39, an amino acid sequence derivative thereof or a salt thereof.

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7. The G protein coupled receptor protein according the group consisting of an amino acid sequence represented by sequence represented by SEQ ID NO: 38, SEQ ID NO: 39, or SEQ SEQ ID NO: 56 and substantial equivalents to the amino acid to claim 6, comprising an amino acid sequence selected from SEQ ID NO: 38, an amino acid sequence represented by SEQ ID NO: 39, an amino acid sequence represented by

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8. The G protein coupled receptor protein according to claims 6 or 7, wherein said receptor is a purinoceptor.

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useful as an immunomodulator or an antitumor agent, in addition to any of claims 6 to 8, wherein an agonist to said receptor is 9. The G protein coupled receptor protein according it is useful in therapeutically or prophylactically.

antagonist to said receptor is useful as a hypotensive agent, treating hypertension, diabetes or cystic fibrosis, and an an analgesic, or an agent for therapeutically or prophylactically treating incontinence of urine.

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coding for a G protein coupled receptor protein of claim 6. 10. A DNA which comprises a nucleotide sequence

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nucleotide sequence coding for the G protein coupled receptor 11. The DNA according to claim 10 comprising a

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protein according to claim 7.

nucleotide sequence represented by SEQ ID NO: 40, SEQ ID NO: 41, or SEQ ID NO: 57. 12. The DNA according to claim 11 comprising a

σ

- to a control sequence compatible with a desired host cell. DNA derived from a G protein coupled receptor protein DNA expression system comprising an open reading frame (ORF) of according to claim 4 or 10, wherein the ORF is operably linked comprising the DNA according to claim 4 or 10; or an 13. A transformant containing a vector
- 5 to 8, which comprises contacting protein coupled receptor protein according to any of claims 14. A method for determining a ligand to the G
- (i) at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of claims 5 to 8, peptide segments or salts thereof, and mixtures thereof,

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and determining whether said compound to be tested bound to the component of (i). (ii) at least one compound to be tested

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- comprises carrying out a comparison between: according to any of claims 5 to 8 with a ligand, which inhibiting the binding of a G protein coupled receptor protein 15. A screening method for a compound capable of
- (i) at least one case where said ligand is contacted with at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of claims 5 to 8, peptide segments or salts thereof, and mixtures thereof,

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and

(ii) at least one case where said ligand together of G protein coupled receptor proteins or salts thereof with a compound to be tested is contacted with at least one component selected from the group consisting salts thereof, and mixtures thereof. according to any of claims 5 to 8, peptide segments or

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- method according to claim 15 or a salt thereof. 16. A compound which is determined through the
- according to any of claims 5 to 8. agonist or antagonist to a G protein coupled receptor protein 17. The compound according to claim 16, which is an

5

the method according to claim 14. according to any of claims 5 to 8, which is determined through 18. A ligand to a G protein coupled receptor protein

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FIGURE 1

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

Primer HS-1 CGTGGCCATCCTGGGCAACACCCTG G C GG CT T C

CGTGGCGGTGCTCGGCAACCTCGTGGT CATTGGCCTGGTTGGAAACATCCTGGT CCTGGGCGTGATCGGCAACGTCCTGGT **GGTGGGCTGGTGGCCAACGCCCTGGT** CGTGGGCTTGCTGGGCAACATCATGCT **GGTGACCATCATCGGCAACATCCTGGT GGTGGCCTGCTGGGTAACTCGCTGGT GGTGGGAGTGCTGGCCAATGCCCTGGT** CATCGGCATGATTGCCAACTCCGTGGT **GCTGGCAGTGGCGGGCAACGTGCTGGT** CCTGGGCATTGTAGGCAACATCATGG1 **AGTGGGCCTCTTCGGAAACTTCCTGGT GGTGGGCTTAGTGGGCAATTCCCTGGT** CTTTGCCATCGTGGGCAACATCTTGGT HUMOP I ODRE RATAADRE01 HUMRANTES **HUMSSTR3X** HUMCSAAR HUMRDC1A RATAZBAR HSBLR1A HUMSOMAT RNU02083 HUMNMBR **U00442** HTRHR HSHM4

FIGURE 2

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 3' SIDE PRIMER

Complementary Sequence TITGCCATCTGCATGCCCCACAAC to Primer HS-2 C TIT C G G G G T T T T

TTTGCCGCCTGCTGGATGCCTTTTACC TITGIGCICIGCIGGAIGCCTITCIAC TTTGCCCTCTGCTGGTTCCCTCTCAAC TTTGCCCTCTGCTGGTTGCCAAATCAC ITTGCCCTCTGCTGCCTGCCCCTACAC TTTGCCCTCGTCTGGTGCCCTCTCAAC TTTGCCCTTTTATGGATGCCCTACAGG TTTGCCATCTGCTGGCTGCCCTATCAC TTTGCCCTCAGCTGGCTGCCGCTGCAT TTTGCCATCTGCTGGCTGCCCTATCAC TTTGCCATCTGCTGGCTGCCCTACCAC TTTGCCTTGTGCTGGCTGCCTTTGTCC TTTGTCATCTGCTGGATGCCTTTCTAC TTTGCTATCTGCTGGCTGCCCTATCAT **TTTGCACACTGGTCGAAGCCAGACAAA** HUMOPIODRE RATADENREC HUMBOMB3S HUMSSTR42 HUMNEKAR RATA1ARA RATNEURA **HUMSRI1A** 58637154 RATGNRHA MUSGPCR HUMSGIR RNCGPCR S46950 543387

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FIGURE 3

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

	CTCCTCGCCATCGCCATTGAGCGCTAT	M31210
	CTGACAGCTATCGCAGTGGACCGCCAC	P30731
	CTCACCTGCCTCAGCATTGACCGCTAC	L09249
	TTGCTGGCCATTGCTGTAGACCGATAC	X59249
	TTGCTGGCTATCACTGTGGACCGCTAC	X61496
	CTCACCTGCCTCAGCATTGACCGCTAC	L04672
	CTCTGCGCTCTCAGTGTGGACAGGTAC	M60786
	ATGACAGCCATTGCAGTGGACAGGTAT	J05189
	ATGACAACTGTGGCCTTTGACAGATAC	X62934
	ATGACCGCCATCGCCGCTGACAGGTAC	X62933
	TTAACAATTCTCAGCGCTGACAGATAC	L08893
	CTCACGGCGCTCTCGGCAGACAGATAC	M73481
	CTCACTGCCCTCAGCGCCGACAGGTAC	M73482
	CTTACGGCACTGTCAGCTGACAGGTAC	M35328
	CTGATGCTCGTGAGTATCGACCGCTAC	X69676
	CTCTGCACCATGAGCGTGGACCGCTAC	D16349
	TTGACCATGATGGAGTGTGACCGCTAC	L11065
	CTCACCATGATGAGCGTGGACCGCTAC	L11064
	Ø	
-	TT GG GT A C	
	CTGACCGCTCTIACIACTGACCGATAT	Primer 3B
	6	
	T T GG GT A C	T CLERCH OX
	CICACCCCTCTTACTACTCACCCATAC	D

S46665

CTGGCTACCATTAGTGCCGACCGTTTC ATCGCCCTCATTGCTCTGGACCGCTGT

TTGGCCTGCATCAGTGTGGACCGTTAC CTGGCCTGCATCAGTGTGGACCGTTAC CTCGTGGCCATCGCACTGGAGCGGTAC CTCGTGGCCATAGCCCTGGAGCGATAC CTGGTAGCCATCTCTCTGGAGAGATAT CTCACGTGTCTCAGCATTGATCGATAC CTCACGTGTCTCAGCATCGATCGCTAC CTCACGTGTCTCAGCATCGATCGCTAC ATTACCTGCATGAGTGTCGATAGGTAC

X65858

M60626

M73969

L04473 M99418 M88096 M91464 M90065 X64052 L32840

U03642

CTCACCGGCCTCAGCTTCGACCGCTAC

Primer 3D

CTCGCCGCTATIAGCATGGACCGITAT

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Primer 3C

CTCGCCGCTATIAGCATGGACCGITAC

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

FIGURE 4

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### FIGURE 5

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 3' SIDE PRIMER

Complementary Sequence TTTACCITCTGTTGGICGCCCTACCACATC to Primer 6A GT T T

Complementary Sequence TICACCITCTGTIGGICGCCCTACCACAIC to Primer 6B GT T T

L11064	TTCGTGGTGCTGGGCGCCCATCCACATC
L11065	TTCATCATCTGTTGGACCCCCATTCACATC
D16349	TTTATCGTCTGCTGGACCCCCATCCACATC
36963X	TTTGTGCTGTGTTGGGTGCCTTTCCAGATC
M35328	TITECCTICTECTEGCTCCCCAACCATGTC
M73482	TTCATCTTCTGTTGGTTTCCAAACCACATC
M73481	TTCGCCTTCTGCTGGCTCCCCAATCATGTC
L08893	TTTGCCCTCTGCTGGTTGCCAAATCACCTC
X62933	TTTGCCATCTGCTGGCTGCCCTACCACCTC
X62934	TTCGCCATCTGCTGGCTGCCCTTCCACATC
J05189	TTTGCCATCTGCTGGCTGCCCTATCACGTG
M60786	TTCGCCCTGTGCTGGTTCCCTCTTCACTTA
L04672	TITGICATCIGCIGGCTGCCCTACCACGTG
X61496	TTTGCCGCCTGCTGGATGCCTTTTACCCTC
X59249	TTTGCCTTGTGCTGGCTGCCTTTGTCCATC
L09249	TTTGCCATCTGCTGGCTGCCCTACCACGTG
P30731	TTTGCCCTCTGCTGGTTCCCTCTCAACTGC
M31210	TTCATCGCCTGCTGGGCACCGCTCTTCATC
U03642	TTTGCCCTGTGCTGGATGCCCTACCACCTG

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FIGURE 6

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 3' SIDE PRIMER

Complementary Sequence TITITCITTGCTGGITTCCCTACCACATG to Primer 6C C T G C

TTCATCATTIGCTGGCTTCCCTTCCATGTT	TTCTTCTTTCCTGGGTTCCCCACCAAATA	TICTICITITCCTGGGTICCCCACCAAATA	TTTTCTTTCCTGGATTCCCCACCAAATA	TICTICCTGTGCTGGATGCCCATCTTCAGC	TICTICCTGTGTTGGCTGCCAGTGTACAGC	TTTTTCTGTGTTGCTTGCCAGTTTATAGT	TTCCTGCTTTGCTGGCTGCCCTACAACCTG	TTCCTGCTTTGCTGGCTGCCCTACAACCTG	TTCTTTATCTTCTGGCTGCCCTATCAGGTG	TITITICTCTGCTGGTCCCCATATCAGGTG
L32840	X64052	M90065	M91464	M88096	M99418	L04473	M73969	X65858	S46665	M60626

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FIGURE 7

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

GTCACCAACITGTTCATCCTCAICCTG

Primer T2A

**RABIL8RSB RATADRA1B HUMSSTR3Y HUMSRIZA** BTSKR **HUMOPIODRE** HUMADRB1 HUMGALARE GTCACCAACGTCTACATCCTCAACCTG GTGACCAACTACTTCATCGTCAACCTG GTCACCAACTCCTTCCTCGTGAACCTG GTCACCGACGTCTACCTGCTGAACCTG CCCACCAACTACTTTATCGTCAACCTG ATCACCAACATTTACATCCTCAACCTG **ACCACCAACCTGTTCATCCTCAACCTG** ACCACCAACCTGTTCATCCTCAACCTG

HUMCCKAR HUMGARE MMSERO HUMD4C HUM5HT1E HUMD18 HUMSHTR GTCACCAACGCCTTCCTCCTCTCACTG GTCACCAACATCTTCCTCCTCTCCCTG GCCACCAACTATTTCCTGATGTCACTT CCCACCAACTCCTTCATCGTGAGCCTG CCTGCCAACTACCTAATCTGTTCTCTG ATGACCAACGTCTTCATCGTGTCTCTG CCCTCCAACTACCTGATCGTGTCCCTG

S57565 RATADRALA

GTCACCAACTATTTCATCGTGAACCTG

CTGACCAATTGCTTCATTGTGTCCCTG

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FIGURE 8

COMPLEMENTARY OLIGODEOXYNUCLEOTIDE SEQUENCE TO 3' SIDE PRIMER

Complementary Sequence AACCCCITCITCTATTGCTTTITCICT to Primer T7A 0000

RATCCKAR HUMGARE **HUMADRB1** MUSGRPBON HUMGRPR S58541 RAT5HTRTC PIGA2R RATALADREC HUMGALAREC HUMSST28A \$59749 HSHM4 RRVT1AIIR MUSSSRI14 HUMSSTR3Y HUMALAADE RNGPROCR S66181 **AACCCCATCATTTATGCCTTTAATGCT** AACCCTATCATCTACCCGCTCTTTATG **AATCCTCTCTTTTATGGCTTTCTGGGG** AACCCCATCGTCTATGCCTTCCGGATC **AATCCTATCATTTATGCATTTCTCTCT AACCCCGTGTGCTATGCTCTGTGCAAC** AACCCCTTTGCTCTTTATCTGCTGAGC AACCCCTTTGCCCTCTACCTGCTGAGC **AACCCCGTCCTCTACGGCTTCCTCTCG** AATCCCATGCTCTACACCTTCGCTGGC **AACCCCATCATCTATTGCTTCATGAAC AACCCCCTGGTCTACTGCTTCATGCAC AACCCCATCATCTACTGCCGCAGCCCC AACCCTCTGTTCTACGGCTTTCTGGGG AACCCGGTTCTCTACGCCTTCCTGGAC** AACCCGCTCATCTACCCCTGTTCCAGC **AACCCCATACTCTACGGCTTCCTGTCG AACCCCATCCTCTACGGCTTCCTCTCC AACCCCATCCTTTATGGCTTCCTCTCC** 

FIGURE 9

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

Primer TM1-A2 TGITGGTTATIGGIGTTGTIGGIAA CC GC C

|GGTGGTGGTGGTGGTGGCAA GGTGCTGGTGGCTGTGATGGGCAA **IGTTCGTGCTGGGCATCATCGGAAA IGATCATTCTTGGTGTCTCTGGAAA** | GGTGCTGGTGCTGTAACAGGCAA **IGTTCATCTTCGGGGTGGTGGGCAA** IGTTCGTGGCCGGTGTGGTGGCCAA TGTTCGTCGTGGCTTGGTGGGCAA | CATCGTGATAGGTCTTATTGGCAA **ICTTTCTGATGAGTGTTGGCGGAAA** | GGTGATCCTGGCTGTGGTGAGGAA IGGTTATCCTGGCCGTGGTCAGGAA TATTCCTTCTCAGTGTGCGGGGAA HUMNEUYREC RATCHOLREC MMSUBKREC MUSGRPBOM BOVEETBR HUMPGE2R HSU11053 MUSBB2R RRMC3RA HUMPIR BTSKR HUMMR

FIGURE 10

COMPLEMENTARY OLIGODEOXYNUCLEOTIDE SEQUENCE TO 3' SIDE PRIMER

Complementary Sequence GCCATIACCITGGACAGATACCGAT to Primer TM3-B2 A G A G

o Primer TM3-B2 A T A C G A G

GCAATAGCTTTGGACCGCTACTGGT GCCATTAGTCTGGACCGCTACTGGT GCACTGTCAGCTGACAGGTACAAA GCCTTTACCATTGAGAGGTACATA GCCATCGCACTGGAGCGGTACAG GCCATCGCACTGGAGCGGTACAG GCCATGACGCTGGACCGCCACCG GCCATCGCCCTGGAGCGATACAG GCCATCTCTGGAGAGATATGG GCCATTGCGGTGGACAGGTACA GCAATTGCTGTGGACCGCTACC GCCATCGCGGTGGACAGATACA GCCATTGCAGTGGACAGGTA MUSALPZADA **HUMADORALX** HUMOPIODRE HUMCCKBGR MUSGRPBOM RATNEURA DOGGSTRN RAT5HT5A RATCCKAR HSTRHREC HUMCCKR MMGMC5R HUMY2R

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

Primer TM3-C2

CATGGCCGTGGAGAGITACITGGC

MMSUBPREC S68242 **HSMRNAOXY** RATCCKAR HUMARB3A HUMOPIODRE CFGPCR4 HUMNK3R HUMHPR HSS316 HUMGALAREC CATATCGCTGGAGAGATACGGAGC CATGTCCCTGGACCGCTGCCTGGC CATTGCGGTGGACAGGTATATGGC CATCGCGGTGGACAGATACATGGC CATCGCTCTGGACAGGTACTGGGC CCTGGCCGTGGACCGCTACCTGGC CATCTCTCGGAGAGATATGGCGC CATGGCCGTGGAGCGCTGCCTGGC CATTGCCCTGGACAGGTACTGGGC ATGTCCGTGGACCGCTACGTGGC TGGCCTTTGACAGATACATGGC

FIGURE 12

COMPLEMENTARY OLIGODEOXYNUCLEOTIDE SEQUENCE TO 3' SIDE PRIMER

Complementary Sequence TTTGCCITCTGCTGGATCCCCAAC to Primer TM6-E2

TTCTTCCTGTGCTGGATGCCCATC	RATCCKAR
TTCATCGTGTGCTGGACGCCTTTC	HSMRNAOXY
TTCGCCGTCTGCTGGCTGCCCCT	MMNPY1CDS
TTTGCCCTCTGCTGGCTTCCCCT	HUMETSR
TTCGCCCCTCTGTGGCTGCCCCT	CFGPCR8
TTCACCCTCTGCTGGCTGCCCTTC	HUMADRBR
TTTGCCCTCTGCTGGCTGCCCCT	HUMA2XXX
TTTGCCATCTGCTGGCTGCCCTA	HUMOPIODRE
TTTGCCTTCTGCTGGCTCCCCAAC	MUSGRPBOM
TTTGCCATCTGCTGGCTGCCCTAC	RATSKR
TTCGCCATCTGCTGGCTGCCCTTC	HUMSUBPRA
TTTGCCATCTGCTGGCTGCCCTAC	HUMNEKAR

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FIGURE 13

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

Primer TM2F18

ARYYTIGCIITIGCNGAY

AATCTGGCGCTGGCTGAC **AACCTGGCCGTGGCTGAC** AACCTAGCCTTGGCCGAC AACCTGGCCTTTGCGGAT AACCTGGCCGTGGCCGAC AACCTGGCCTTGGCCGAC AGCCTCGCAGTGGCCGAC AATTTAGCACTGGCTGAC **AACCTGGCCGTAGCCGAC** AGCTTGGCTGTGGCTGAT AGCCTGGCAGTAGCTGAT AACCTGGCCTTAGCCGAT HUMINTLEU8 HUMA1AADR HUMEL 4REC HUMANTIR HUMNEKAR HUMIL8RA HUMSOMAT HSTRHREC HUMFMLP HSU07882 HUMTSHX HSDD2

( R = A or G, Y = C or T, N = A, C, G or T, and I = Inosine )

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FIGURE 14

COMPLEMENTARY OLIGODEOXYNUCLEOTIDE SEQUENCE TO 3' SIDE PRIMER

Complementary Sequence TTYNYNNTNTGYTGGITICCI to Primer TM6R21

TTCACCCTCTGCTGGCTGCCC TTTGCCATCTGCTGGCTGCCC TTTGCTCTTTGCTGGTTCCCT TTCATCATCTGCTGGTTTCCC TTCCTGCTTTGCTGGCTGCCC TTCGTGCTCTGCTGGTTCCCT TTCGCCATCTGCTGGCTGCCC TTCGCCATCTGCTGGCTGCCC TTTATCATCTGCTGCCC TICTICATCIGITEGITICCC TTCATCTTCTGTTGGTTTCCT TTCATCATCTGCTGGCTGCCC TTTGCAGTCTGCTGGCTCCCT TTGCCCTCTGCTGGCTGCCC TCTTCATCTGTTGGTTTCCC TTCATCATCTGCTGCTGCCC TTCGTGCTCTGCTGGATGCCC HUMNEUYREC HUMHI SH2R HUMA1AADR HUMSUBPRA **HUMPFPR2A** HUMNEKAR **HUMSHT1DA HUMETN1R HUMIL8RA** HUMNKIRX **HUMSSTR3X** HUMNMBR HUMFMLPX **HUM2XXX HUMBK2A** HSDD2

'(Y = C or I, N = A, C, G or I, and I = Inosine)

TTTTTCTGTGTTGGTTGCCA TTTGTGGTCTGCTGCCC

HUMCCKR HSNEURA

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FIGURE 15

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

Primer S3A GCCTGITIAIGATGAGTGTGGAIAGIT
C G C TC C

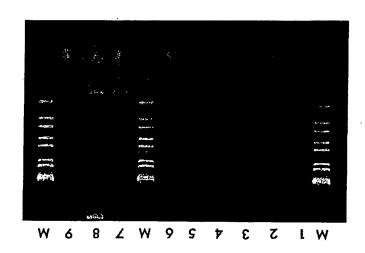
HUMBK2A S44866 S67127 S70057 HUMGALAREC HUMNEKAR HUMIL8RA HUMFSRS HUMGRPR **HSNEURA** HUMANTIR HUMC5AAR CCATGACCGCCATTGCTGCCGACAGGT GCCTGACAGTCATGAGCGTGGACCGCT CACTCACGGCGCTCTCGGCAGACAGAT TGTTGGCCTGCATCAGTGTGGACCGTT ACGTGGCCAGCCTGAGTGTGGAGCGCT **GTCTATGTGCTCTGAGTATTGACAGAT** GCCTCGTGGCCATCGCACTGGAGCGGT TCCTGATGCTGGTGAGCATCGACCGCT ACCTCTGCGCTCTTAGTGTTGACAGGT CCCTGGCCGCGATGTCCGTGGACCGCT TACTCACGTGTCTCAGCATTGATCGAT TCCTGGCCACCATCAGCGCCGACCGCT

COMPLEMENTARY OLIGODEOXYNUCLEOTIDE SEQUENCE TO 3' SIDE PRIMER

FIGURE 16

Complementary Sequence TGGITICCCTACCACITIATCAICATC to Primer S6A T T GG GT

HUMNEKAR	HUMIL8RA	HUMFSRS	HUMGRPR	HSNEURA	HUMBK2A	HUMANTIR	HUMC5AAR	544866	567127	\$70057	HUMGALAREC
TGGCTGCCCTACCACCTCTACTTCATC	TGGCTGCCCTACAACCTGGTCCTGCTG	TGGCTGCCCTTCTTCACCGTCAACATC	TGGCTCCCCAATCATGTCATCTACCTG	TGGACTCCGTTCCTCTATGACTTCTAC	TGGCTGCCCTTCCAGATCAGCACCTTC	TGGATTCCCCACCAAATATTCACTTTT	TGGTTGCCCTACCAGGTGACGGGGATA	TGGCTTCCCCTTCACCTCAGCAGGATT	TGGTTCCCTCTTCATTTAAGCCGTATA	TGGTTGCCAGTTTATAGTGCCAACACG	TGGCTGCCGCACCACATCATCCATCTC



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CGCCACTGGCGTTCGGTGCTGTGTGCGGGGGGGGTCGAGCGTCGA

ACGAGCTCTTCATGCTGAGCGTGCCCTTCGTGGCCTCGTCGCCCTC

DOODATOODETOOACTOTTOOATOTAOAAOOATODOOAAADTADAAO

CAAGATGAAGACGCTACCAACATCTACCTGCTCAACCTGGCGTAGCCG

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09

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TAMOSMUH

S-TT-82A

TAMOSMUH

S-7T-82A

TAMOSMUH

S-7T-82A

TAMOSMUH

2-7T-82A

CAGTGTCCACACCCGGCCTGGTCGGCAGTCTTCGTGGTCTACACTTTCCT 

CAGTGGCCACACCCGGCCTGGTCGGCAGTCTTCGTGGTCTACACTTTCCT

GCTGGGCTTCCTGCTGTCCGTGCTGTCCATTGGCCTGTGCTACCTGCTCA 

GCTGGGCTTCCTGCTGCCGTGCTGGCCATTGGCCTGTGCTACCTGCTCA

TCGTGGCAAGATGCGCGCCGTGGCCCTGCGCGCTGGCAGCAGCGC

AGGCGCTCGGAGAAAAATCACCAGGCTGGTGCTGATGGTCGTGGTCGT 

AGGCGCTCGGAGAAGAAATCACCAGGCTGGTGCTGATGGTCGTGGTCGT

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,	HUMDRO5A	57-A-2	1		HUMORO5A		57-A-2	i		HUMURUSA		7-W-1C	3-3-3		HUMDRD5A		57-A-2	•		HUMDRD5A		57-A-2	•		HUMDRUSA		57-A-2			HUMURU5A		57-A-2	
714	CGGCTGG	GGGGCTGG	w	664	TICATICO	::	TICATIC	N	614	TOGCCITO		Treectife.		564	CIGGGCC	::	CIGGGCC		514	TOCTOCAL		TOCTOCAL		464	ACTIGOOD	:::::::::::::::::::::::::::::::::::::::	ACTOGOCO		_	GIGGCCC	X: ::	GIGGGCA	
724	CEGGCTIGGACCTIGCCAAA	GGGGCTGGAACCTGCCAAA	310	674 684	BGTCCAGCTCA		RELICEMENTON	260 270	_	GICATGGICGG		SELECTION TO SELECTION OF SELEC	210 220	_	VICTOCIOCOCO		ICTCCAGOCCC	160 170	524 5	TOCCTOCATO		TIGOCTICCATIC	110 120	474 4	TITIGGAGCGIT		TITIGGAGCGIT	60 70	424 4	IGCIGGICATOC	::	TOCTGGGCAACC	10 20
				4 694	CTOCACAGG		CTGGGACAGGG	280	_	CTGGCATGGAC		CIGGCAIGGAC	230	•	LICCOCIACAAC	:::::::::::::::::::::::::::::::::::::::	TTCCGCTACAAC	0 180	534 544	TOMOCTOTICX		TEMACCIGICA	0 130	484 494	CIGOGAGGICI		CIGCGACGICI	0 80	434 444	-CCTGGAAGGC	::	TOCTIGENAGEC	0 30
				704	TICATICOGGICCAGCICAACIGGCACAGGGACCAGGCGGCCTCTIGGGG		TICATICCGGICCAGGICAACIGGGACAGGGACCAGGCGGCICTIGGGG	290	654	TOGCCTTGGTCATGGTCGGCCTTGGCATCCTCATCTCC		INSCRIPTION OF THE PROPERTY OF	240		CTGGGCCATCTCCAGGCCCTTCCGCTACAAGCGCAAGATGACTCAGCGCA	*** *** *** *** *** *** *** *** *** **	CTGGGCCATCTCCAGGCCCTTCCGCTACAAGCGCAAGATGACTCAGCGCA	190	554	TIGOTICCACTICCCATICCTIGAACCTIGTIGCGTCATICAGCGTIGGACCGCTA	### ### ### ### ### ### ### ### ### ##	TOCTOCACTOCCTCCATCCTGAACCTGTGGGTCATCAGCGTGGACCGCTA	140	504	ACTGGCCCTTTGGAGCGTTCTGCGACGTCTGGGTGGCCCTTCGACATCATG	20 00 00 00 00 00 00 00 00 00 00 00 00 0	ACTOSCCCTTTOGAGCGTTCTGCGACGTCTGGGTGGCCTTCGACATCATG	90	454	GIGGCGCIGCIGGICATGC-CCTGGAAGGCAGTCGCCGAGGTGGCCGGTT		GIGGGCATGCIGGGCAACCICCIGGAAGGCAGTCGCCGAGGTGGCCGGTT	<b>4</b> 0
					TIGGGG		TIGGGG	300		ATCTCC	::	ATCTCC	250		AGCGCA		AGCGCA	200		VICTORY A	::	CCGCTA	150		ATCATO		CATCATG	100		CCGGTT	::	CCGGIT	50

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A58-SP6

HUMSOMATA

A58-SP6 HUMSOMATA

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CTITICCCTCTGCTGGTTGCCTCTCCAC CTTTGTGCTCTGCTGGATGCCTTTCTAC 

A58-SP6 HUMSOMATA

HUMSOMATA

FIGURE 19

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FIGURE 24



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FIGURE 25

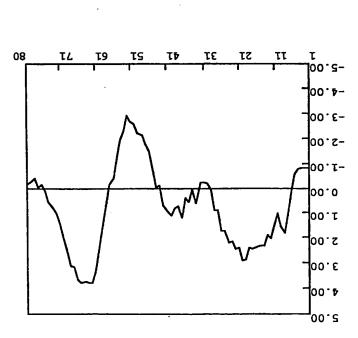


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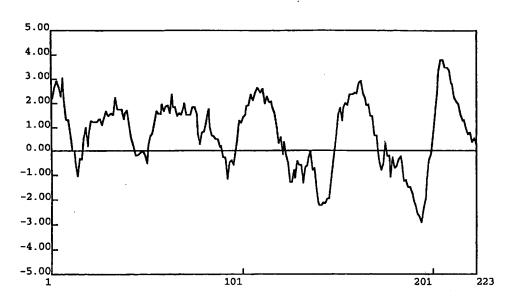
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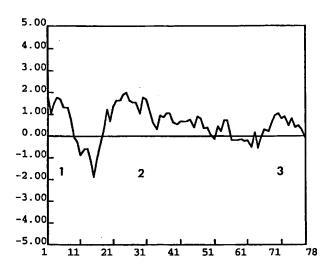
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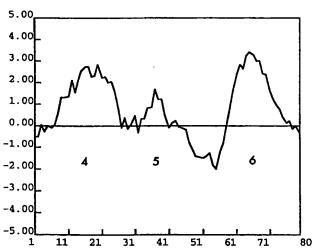
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FIGURE 33

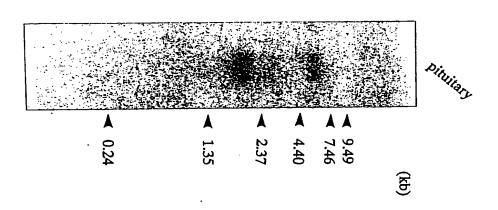
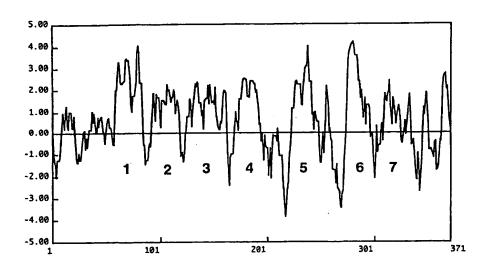


FIGURE 36



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FIGURE 37

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A46226 1	RYLAVVHPTR	SARWETAPVA	RTVSAAVWVA	SAVVVEPVVV	FSGVPRG-	5 0
JN0605 1	RYVAVVHPLR	AMTYMRPS VA	KLINLGVWLA	SLLVTLPIAL	FADTRPARGG	5 0
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p34996 51	KTTTCYDT	TADEYLES	VYSMCTTVFM	FCIPFIVILG	CYGLIVKALI	100
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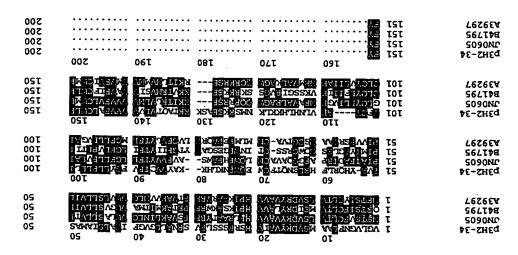


FIGURE 42

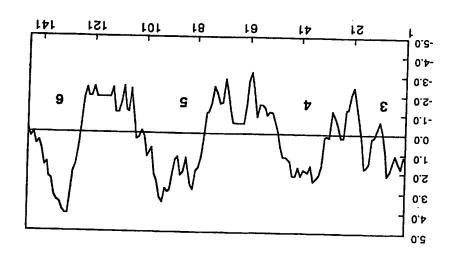


FIGURE 44



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CTOOGENCTTGCAGCACCOCCTCCTCTTTAGCCAGGCCAGGACGACGACTGTGATGATGGGCACAGGGG
GTGGTLTTCCAGGCTTTCTTGCGGGTTGCGGGAGTACTAGTTGGAGACGCGCGCG
CTGTCCTGGGCCACTCGGTGATCCTAGAGCCAGTTTTCCCTGGCTGG
GOCTICCGGTCCATTGCACAGGGCCCCAAGGGGTTATCCCAGTAATGATGATGAAGCTGATGATGAACTTCCACTGCAAGAGACTTCCAGTGCAAGAGAGAACTTCCAGTGAAAAAACAACAAGAAGAAAAAAAA
AGTGAAGGGAATGGGAACCAGAGCGGCAAGCCGGAGTCCAAGCCGCTCTTCGGCATGGCATGGAG SerGluGlyAsnGlySerAspPrGluProProAlaPrGluSerArgProLeuPheCly1leGlyValGlu
AACTICATRACOCTIGATAGNOTTAGOCCTGATTATAGOGAJOGOCTGCTGGGCAACAGCCTGGATAAACAC AsnPhellemateuvalvalvalphegiyleuilePhealameatgiyvalleucilyasnSerleuvallieftr
GTGTTGGGCCCGAGAAACCAGGAAACCGGGCAACAACAACTAGTTAATCCTCAATCTGAGAATGGA Vallewalaakg8eriy8ProGlyfy8ProArg8erThrThrAsmLewPheillefawasmLewSerIleAla
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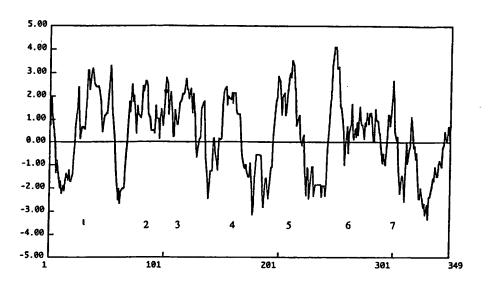


FIGURE 48

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MOUSEGALRECE HUMGALAMI	151 WALLGVOOR IN	ALSIAMASPU	180 AYHORLEH- AYHOGLEH-P	190 DSNQTFCWEÇ ASNOTFCWEC		200 200
MOUSEGALRECE HUMGALAMI	201 VVCTFVFGYL 201 VVCTFVFGYL		230 AKVLMHLIKE AKVLMHLIKE	240 JANNISKKSEA JANNISKKSEA	250 SKKKTAOTVI SKKKTAOTVI	250 250
MOUSEGALRECE HUMGALAMI	260 251 /VVVVFGISE 251 /VVVVFGISE		280 EFCAFPLTPA EFGVFPLTPA	290 SFEFRITANC SFEFRITANC	300 LAYSNSSVNP LAYSNSSVNI	300 300
MOUSEGALRECE HUMGALAMI	310 301 HYAFLSENF 301 HYAFLSENF	320 ЗКАЧКОУБКО ЗКАЧКОУБКО	330 VCDESPREE IRKOS-ILSO	340 PKENKSRMDT PKENKSRIDT	350 PPSTNCTHVX PPSTNCTHVX	350 350
MOUSEGALRECE HUMGALAMI	360 351 351 X	370	380	390	400	400 400

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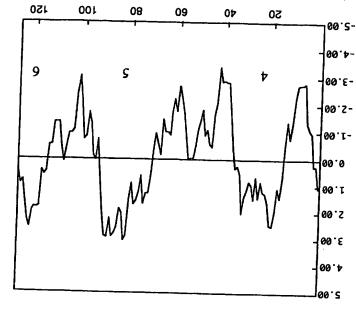


FIGURE 50

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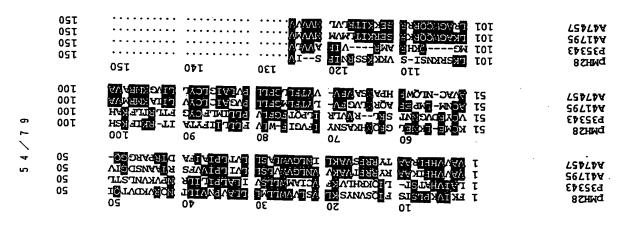


FIGURE 54

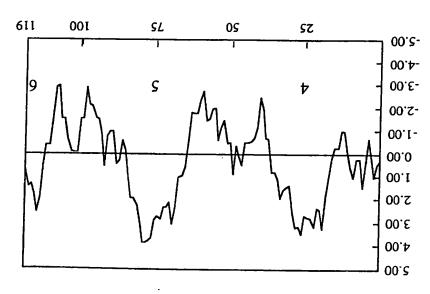


FIGURE 53

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FIGURE 55

GCC ACC AAC GTG TTC ATC CTG TGT CTG GGG GAC CTG GCT GCC CTG ACC CTG Val Asp Leu Leu Ala Ala Leu Thr Leu

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ATG CCT CTG GCC ATG CTC TCC AGC TCC GCC CTC TTT GAC CAC GCC CTC TTT GGG

ATC CTC TCG GTG TCC GCC ATC AAT GTG GAG CGC TAC TAT TAT GTG GTC CAC CCC

ATG CGC TAT GAG GTG CGC ATG AAA CTG GGG CTG GTG GCC TCT GTG GTG GGC

Het Arg Tyr Glu Val Arg Met Lys Leu Gly Leu Val Ala Ser Val Leu Val Gly

TOP GLU GLU GLY Pro Pro Ser Val Pro Pro Gly Cys Ser Leu Gln Trp Ser His

AGT GCC TAC TGC CAG CTT TTC GTG GTG GTC TTC GCC GTC CTC TAC TTC CTG CTG

Ser Alo Tyr Cys Gin Leu Phe Vol Vol Vol Phe Alo Vol Leu Tyr Phe Leu Leu

FIGURE

GGA GGA CAG TIC CTG CTC TGT TGG TTG CCC TAC TTC TCC TTC CAC CTC TAT TC1 GIy Gly Gly Gln Phe Leu Leu Cys Trp Leu Pro Tyr Phe Ser Phe His Leu Tyr ACC ACC CCT CAC CGG ACG TTT GGC GGA GGG AAG GCA GCA GTG GTC CTC CTG GCT

Thr Thr Pro Nis Arg Thr Phe Gly Gly Gly Lys Ala Ala Vol Vol Leu Leu Ala 



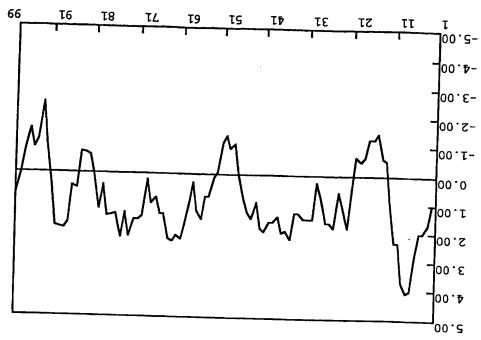


FIGURE 58

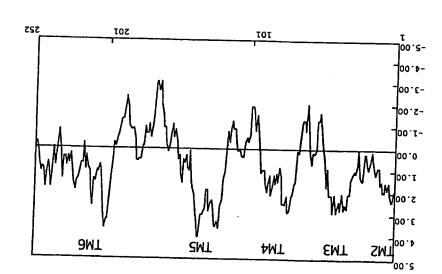


FIGURE 57

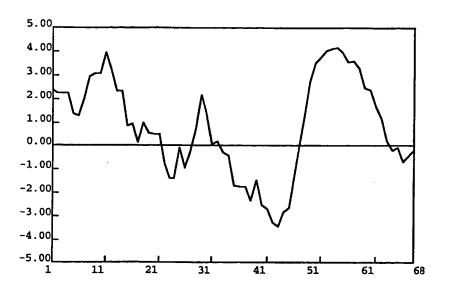


FIGURE 60

p19P2 S12863	10 20 30 40 50  1 VGWVSWLIV LVHARVRRLH SVINFOLSH ALSDVINCTA GVSLILAVAF 1 LGVSGRABI IIILKQKEMR SVINITIVSH SFSDLIVAVM SLEFIFVNIL	50 50
p19P2 S12863	51 EPRGETYFOOG LCHLVFFLOP VIVYESVETT TTIAVORYVV LVHELRRRI- 51 MDH-WVFGET MCKLNPEVOC VSITVSLESE VLIAVERHOL IIN RGWREN	100 100
p19P2 S12863	110 120 130 140 150 01 NRHAYIGITV IWVLAVASSL PFVIYQILID EPFQNVSLAA FKDKYVCFDK	150 150
p19P2 S12863	160 170 180 190 200 51GLELV HYPPLEVIL LS VRVSVKERNE VVPGCVTQSQ 51 FPSDSHRESY TITLLVEQYF GPLCFIFICE FKIYIREKRE NIMMOKIRDS	200 200
p19P2 S12863	210 220 230 240 250 01 ADWDRARR TFC LLVVVV VEAICVLEVY 01 KYRSSETKEI NVMLLSIVVA -FAVCSILELT	250 250

220 220	sz	 5 <b>4</b> 0	SXX	VAAAE <mark>MT</mark> CAIT VAAAE <mark>MT</mark> CAIT	SIO RRTFCLLW RRTFCLLW	207 207	Ď€3-5\Ď€Ţ-70 Ď7∂Ъ5
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720 720	T20	TYDOMICE TYO	130  EVHYTHVAA	120 LALIVESTVMI	OII AIVAYASJAJI	101 101	DG3-5\DGT-10
00T 00T	1001 - Тяяяланул гіяяяданул	<b>00</b> VVYADVAITI VVYAGVÁÍŤI	80 VIVYVSVFTL ITHVSVFVTV	ГСНГЛЕБГСУ ГСНГЛЕБГСУ 10	EPRGNVFCGC	TS TS	PG3-2\pGT-10
0S 0S		40 ALSDVLMCTA ALSDVLMCTA	MALMETICHT MALMETICHT 30		10 Venverviusv Venveriuusv	T T	pg3-2/pg1-10

FIGURE 61

FIGURE 62

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CIG TGT GTC ATC GGG G

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FIGURE 63

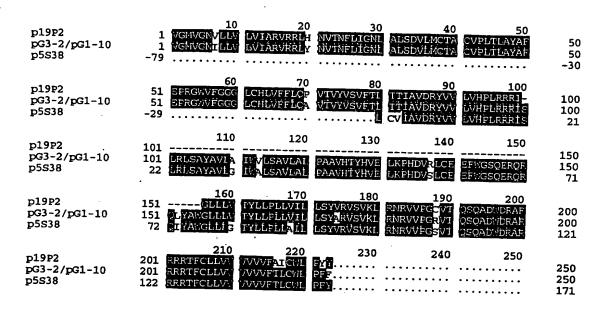
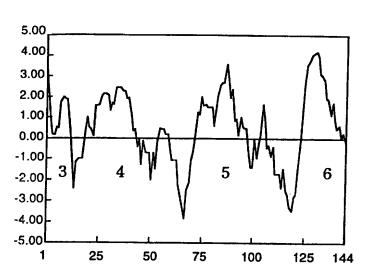


FIGURE 64



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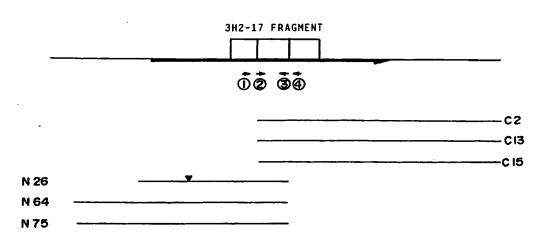
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FIGURE 67



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FIGURE 69

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	61 CACTTCCCCCACCACCTCTACCATCACATTTCAACCAAC	121 GTATACTOGGTGGTGGTGGTCGCCCGCTGAACACTCGGGTGATTGCCGAATC 31 ValiyrServalValLeuValValGiyLeuProleuAsmileCysValIlaAlaGinIla	181 TOCCATOCOCOCOGALCCTOACCOCTOCOCTOTOTACCTGAACCTGGAACTGGCG 51 Cysalaserkgargfirleuthrayseralavaltyrthreuasneualaeaala	241 GACCIGATGTATGCCTGTTCACCCCTACTTATCTATACTACGCGGGGGGCGCCT 71 AspleatherTytalacysSerLeuproLeuleutletyfastfyfalacygglyasphis	301 TOSCCCTTCGGAACCTCGCCTCCCTTTGTAGGCTTCCTCTTCTATGCATCTAGAT 91 TrpProPhedlyAspLeualaCysArgPheVallargPhasLeubheTyrallaNsstamHis	361 GCOAGCATCTTGATACTTGATAGGTTCCAGGCTTAGCTGGGGAATCTGCGACCC	421 CTGCCTTCCTGCCACAAGCGTGGAGTGCTGCTGCGTGGGTGAGTGTGTGGAGTGTT 131 LeualaSerTtpHisLysAtpGlyGlyAtgatgalaalattpVallyValValVal	481 TGGCTGGCTGTGACAGCCCAGGCCCGCGAGGCGAGTCTTGCTGCCAGGCAACCAGCAACCAGCAACAAAAAAAA	541 CCCAACCCACTGTGTGCTBAGACCCAACCAACGTGATAATAATAACTGACC 171 AFBARAYTHYVAIQYAAFDAASETSOPTOIleLeuserTHAAFJYALeubro	601 TATOGTATOCCCTCACOGTCATOGGCTTCTTOCTOCCTTCATAGCCTTACTGGCTTGT 191 TyrglymetalaleuthrVallleOlypheleuteuptophelleAlaleuteualacys	661 TATTGTCGCATGGCCCCCTGTGTCGCCAGARGCCCCAGCAGARCTCTGTGGCCCA 211 TycCygArgherllaArgArgLeuCyaArgGLAASpClyProAlaGlyFroMallAgGla	721 GACCGCCCACCAACCACCCTCCAATGCCTGTGCTGCCACCTCTCTTTGCCATCAC 231 GLAKTGATSETLYSALAALAAKTGKETALAVAIVAIAALAALAVAIPHEALAIISSET	781 INCCROCCITICO CANTOACO AGACCIACITO CONTOCO CONTOCO CONTOCO 251 Prefebro Problema Infrata y Translator Alabara Arabara Produya 1	841 TCTTGCCCTGTGCTGGAGCTTGCTGCTGCTACAAAGGCACTGGGCCTTGGGGT 271 SerCysProvaLLeuGluthrPheAlaAlaAlaTyrLysGlyThrArgproPheAlaAser	901 GTCHACHGTGTTGTGGACCCCATTCTTCTACTTCACACAGAGAGTTCGGCAGCAA 291 ValkanServalleumspptollalæuphetyrphettuglaglalyaphetggla	961 CCCCACGARCTCTTAAAAAAACTCAAAACAAATGAAAAAAAAAA	

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912 V73 339 Pro Leu 458 459 468
GGC CTC ATG GCC ATG GCC TAT TTC CAG
GIy Leu Met Ala Met Ala Tyr Phe Gln 477 ATC TTC CGC : 꿁 \$ ; 486 AAG CTC TGG ļ ۲۶ Leu Trp

SAC GAC CTG TAC CCC AAG ATC TAC CAC AGC TGC TTC ATT GTC ACC

Asp Asp Leu Tyr Pro Lys The Tyr His Ser Cys Phe Phe Ile Vol Thr Tyr Leu 1AC CT6

S 333 342 351 360 369 378
GAG CTG GCC AAC CGC CCC CTC CTC TCT GTC TGT GAG CGC TGG GCA

Glu Leu Ala Asn Arg Thr Arg Leu Leu Ser Val Cys Asp Glu Arg Trp Ala 324 AGC GTG CTG --- ---Ser Val Leu

TTC AAG AGC ACT GCC CGC GCC CGC GGC TCC ATC CTC GGC ATC TGG GCC GTG

Phe Lys Ser Thr Ala Arg Arg Ala Arg Gly Ser Ile Leu Gly Ile Trp Ala Val

Thr Leu Ser Ser Ile Ala Leu ACT CTC AGC TCC ATC GCC CTG į 189 198 G GAC CGC TGG TAC GCC / Asp Arg Trp Tyr : Ala Ile Cys His Pro 98 207 10 ATC TGC CAC CCG C 216 --- ------ ---

CTC TGC 117 126 135 144
TGC AAG GTC ATC CCC TAT CTA CAG GCC GTG TCC GTG TCA
Cys Lys Val Ile Pro Tyr Leu Gln Ala Val Ser Val Ser 

CTG CCG GCC AGT CTG CTG GTA GAC ATC leu Val Asp Ile Thr Glu Ser Trp Leu 

Ala Asp Val Leu Val Thr Ald The Cys

SCC ACC AAC GTG TTC ATC CTG TCA CTG GCC GAT GTG CTG GTG ACA GCC ATC TGC

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FIGURE 72

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PZUR\_HOUSE
PZYR\_CHICK 75+13, CODING PZUR\_MOUSE PZYR\_CHICK 75+13,CODING PZUR\_HOUSE PZYR\_CHICK 75+13, CODING PZUR\_HOUSE PZYR\_CHICK 75+11, CODING PZUR\_HOUSE PZYR\_CHICK 351 351 351 200 2 2 2 2 2 2 2 2 2 2 2 2 \$25E 200 151 151 151 151 1010 ងកដ ACPTIENTS SERVICE HISTORY SERV - September Statement Beducker Statement State 260 270 280 270 280 CONTROL TO CO CHARLES IN PARTY LIES STATES THE TRANSPORT OF THE TRANSPO 10 20 30 30 AVERTAGE TO THE TOTAL STATE OF THE TOTA LSVSSDOSRR (QNGDTSL... 290 100 C 51211 100 C 51211 LIR-VIII. A TENTAL STATE OF THE STATE A COLUMN 250 - YV-\$ 50 888 350 888 250 250 200 120 120 120 120 888

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FIGURE 73

Gly Arg Gln Ile Pro Gly Thr Thr Ser Ala Leu Val Arg Asn Trp Lys Arg Pro

603 612 621 639 639 648 GCC CGC GCC TTC CTG GCC GAG GTG AAA CAG ATG CGA GCC CGG AGG AAG AAG GCC

AAG ATG CTG ATG GTG GTG CTG GTC TTC GCC CTC TAC TAC CTG CCC ATC AGT

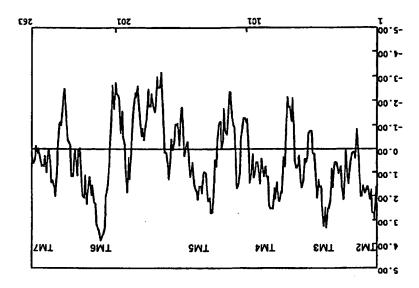
Lys Wet Leu Met Val Val Leu Leu Val Phe Ala Leu Cys Tyr Leu Pro Ile Ser

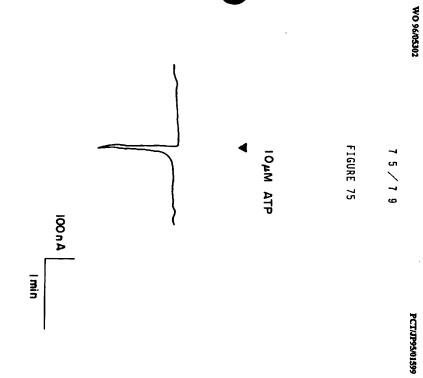
Ala Arg Ala Phe Leu Ala Glu Val Lys Gln Met Arg Ala Arg Arg Lys Thr Ala

6CC 6CC AAT CCC CTC CTC TAC TCC TTC CTC CCT 3'

Ala Ala

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h3H2-17(5	-31	1	10	20	30	40	50	
p3H2-17 (5		ī	GIGGGCCIGG	TGGGCAACAT	CCTGGCTTCC	TGGCACAAGC	GIGGAGGICG	50 50
h3H2-17 (5	-3)	51	60	70	8.0	90	100	100
p3H2-17(5	`)	51	CCCTGCTGCT	TGGGTAGTGT	GTGGAGTCGT	GTGGCTGGCT	GTCACICOCO	100
h3H2-17(5 p3H2-17(5		.01 .01	110 IGNGC TGCG IGNGC TGCG					150 150
h3H2-17(5 p3H2-17(5		51 51	160 Acusticies Acusticies Acusticies	170 ATCACCICAG ACCACCIGAG	180 CCOP COPTICATO CCA COCATO	190 Erde Gesche Erdersche	200 ACEATATEC GEFACCITO: C	200 200
h3H2-17(5 p3H2-17(5			210 SPAN CAN SPAN CAN	220 Corpus Cre Coccus Cre	230 TERROGETT TERROGETT	240 C 100 (C) C 100 (C)	250 TEIGCTEOIC TEICATAGOST	250 250
h3H2-17(5			Percent 16 Activat 16	270 CMCTURETS THE WALLS	280 Chica Total Africa Egiv	290 COLIGIGICG	300 CCAGGATGGC	300 300
h3H2-17(5- p3H2-17(5)		01 01	310 CCAGCAGGTC	320 CTGTGGCCCA	330 AGAGCGCCCC	340 AGCAAGGCGG	350 CTCGTATGGC	350 350
h3H2-17(5- p3H2-17(5		51 51	360 TGTGGTGGTG	370 GCAGCTGTCT	380 TIGCCCICIG	390 CTGGCTGCCT	400	400 400

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1 TGACTCCCTGAACATAGGAAACCCACGTGGGCATGGGAATGGGAATGGCAACAGGC 1	61 CAGSCTCTGGGACCCACCACCTGTGTCTACCGGAGAACTTCAAGCAACTGCTG 8 G1mA1aLeuG1yLeuProProThrThrCygVa1TyrArgGluAsnPheLysGLnLeuLeu	121 CTGCCACCTGTGTATTGGGGGGGGGGGGGGGGGGGGGGG	181 ACCCAGATCTGCACGTCCCCCGGGCCCTGACCCGCAGGGCCGTGTACACCCTAAACCTT 48 ThtGlailleCysThtSetArgArgAlaledThtAaygThtAlaValTyTftTeLeuAsmLeu	241 OCTOTOSCTOACTOCTATATOCCTOCTOCTOCTOCTOATCACAACTAGOACTAACAACTAAAAAAAAAA	301 GOTGATCACTGGCCCTTTGGCGACTTGGCCTGGTGCGCTTGCTT	361 ACCTGCACGCAGCATCTTCTTCTCTGCATCACTTCCAGCACCAGCACTTCAGCACTTCCAGCACTTCCAGCACTTCCAGCACTTCCAGCACTTCCAGCACTTCCAGCACTTCAACTACTACTACTACTACACTACACTACACTACT	421 TOCCACCOGCTOSCCCCTTGGCACAAAGGTGGGGGCCCCCGGGCTGCCTGGCTAGTGTGT 128 CysHisFroLewAlaProTrpHisLysArgGlyGlyArgArgAlaAlaTrpLeuValCys	1 GTAACCGTGTGGCTGGCCGTGACACAGTGCCTGCCCGAGCGACTTCTGCCTGC	1 GCOATCCAGOGRACCGGACTGCTGTGTGTGAGCTGCCGCCTGGCCGACCGAC	1 TATATOCCCTATOSCATOTICACTOTICATOCCTOCTISCTISCOCTITICATOCCTOCOCTIS B Tytheefrotyrglymetalalacutatomallacut	1 CTOSCCTOCTACTOCTCCCTCCCCTCTCCCCCTCTCCCCACACGCCCGCCAGAGCCT B LeuklaCysTyrCyslaulaulacysatglandpcJyproklaglupro	1 GTOSCCCAGGACCOGCOSTIGSCAAGGCGCCCCACATGGCCCGTGTTGGTGCTGCTTGCTTG	. GCCATOACCTICCTOCCTITICACATCAACAAGAGCCTAACCAGTGGCCTGGACGAGGAALAAAAAAAAAA		TITOCOAGIOCOACAGOGISCIOGACCCATCCICTICTACITCACCAGAGAGTIC PheAlaSerAlaAsnSerValLeuAspProIleLeuPhefyrPhefhighlaysiysPhe	COCCOCCACCACATGACTCCTACAGAAACTCACAGCCAAATGGCAGAGGGCAGGGTCCC AFGATGATGPTOHLSGLULeuLeuGlaLysteuThtAlaLysTtpGlaArgGlaGlyArg	TGA
		н	-	ñ	m ~	ÄÄ	128	148	541 168	601 188	661 208	721 228	781 248	841 268	901 288	961 308	1021 328

human prino, mouseFULL3H2		50 50
human prino, mouseFULL3H2	50 70 80 90 100 51 ICTERPALTE TAVYTINIAL ADLLYACSLE LLYNYADGI HUPFGDFACE 10 51 ICASERILTE SAVYTINIAL ADLMYACSLE LLYNYARGI HUPFGDFACE 10	
human prino, mouseFULL3H2	110 120 130 140 150  101 L'ARLEYANL HGSILFLTCI SFORYLGICH PLASHKRGG REARLYCUT 15  101 FURFLEYANL HGSILFLTCI SFORYLGICH PLASHKRGG REARLYCUT 15	
human prino, mouseFULL3H2	150 170 180 190 200 151 ANEAVER SET STATES SERVING SPECIAL STATES STATES SERVING SPECIAL STATES SERVING SPECIAL STATES SERVING	•
human prino, mouseFULL3H2	210 220 230 240 250 201 FLUERALLE SYCLLEGIC RODGENERVA DERIC RAARU AVVALAFAI 250 201 FLUERALLE SYCRUFRIC RODGENERVA DERIC RAARU AVVALAFAI 250	
human prino, mouseFULL3H2	260 270 280 290 300 251 SELPEHITKI KYLAVISTEC VP. 1 TLEASE ANYKGIRPEA ALSVLOPIL 300 251 SELPEHITKI KYLAVISTEC SSETTLEBEE ANYKGIRPEA VISVLOPIL 300	
human prino, mouseFULL3H2	310 320 330 340 350 301 37FT(KEFF) REPOSLORET ANYORGER*	

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and mailing address of the ISA European Patent Office, P.B. Still Patentham 2 N.L 2250 P.V. Styring N.L 2250 P.V. Styring Tel. (+ 3) - 70) 340-2504, Tx. 31 651 epo al, Face (+ 4) - 70) 340-2504, Tx. 31 651 epo al,	18 December 1995	of the actual completion of the international search	Special categories of cited documents:  Ye document defining the general state of the art which is not considered to be of particular relevance.  E' earlier document but published on or after the international filing date.  L' document which may throw doubts on priority claim(i) or which is cited to establish the publication date of another citation or other special reason (as specified).  O' document referring to an oral disclosure, use, established or other means:  P' document published pror to the international filing date but the priority date claimed.  A:	he continuation of box C.	-/-	EP,A,O 578 962 (AMERICAN CYANAMID CO) January 1994 see example 2	WO,A,92 01810 (LERNER MICHAEL R ;LE ETHAN A (US)) 6 February 1992 see abstract; claims 1-17	see the whole document	MOLECULAR ENDOCRINOLOGY, vol. 5, January 1991 pages 1331-1338, M.T.HARRIGAN ET AL. 'Identification of a gene induced by glucocorticoids in murine T-cells: a potential G protein-coupled	Clution of document, with indication, where appropriate, of the relevant passages	C. DOCUMENTS CONSIDERED TO BE RELEVANT	Electronic data base consulted during the international search (name of data base and, where presental, search terms used)	Documentation searched other than managem documentation to the extent that make documents as a solution in the deleteration to the extent that make documents as a solution in the deleteration to the extent that make documents as a solution of the extent that make documents as a solution of the extent that make documents as a solution of the extent that make documents as a solution of the extent that make documents as a solution of the extent that make documents as a solution of the extent that make documents as a solution of the extent that make documents as a solution of the extent that make documents as a solution of the extent that make documents as a solution of the extent that make documents are a solution of	Minimum documentation searched (dassification system followed by classification symbols) IPC 6 C07K C12Q	B. FIELDS SEARCHED	g.	A CLASSIFICATION OF SUBJECT MATTER IPC 6 C12N15/12 C07K14/705 C12Q1/68	
Authorized officer Gurdjian, D	05.01.96	Date of mailing of the international ser	later document published after the international filing data or priority data and not in conflict with the application that that to understand the principle or theory underlying the internation of published in the priority of the considered in ord or caused be considered novel or assess the considered to be involve an inventor. It also alone the considered to purchase reference to purchase reference the diamed inventor as consistence to purchase reference the diamed inventor as consistence to considered to involve an inventor as consistence of purchase reference with once more other such documents is combination being obvious to a person delited in the art.	Patent family members are listed in ansex.		CO) 19	;LERNER		on of a murine upled	ant passages		nd, where practical, search terms used)		symbols)		toon and IPC		ייי ארוייר אין
		search report	rustional filing data to the application but for a preplication but for a but a but defined invention be connected to tennest it taken alone tennest it taken alone daimed invention daimed invention to a person delited tamily	n anner.		1-3	14-18	14-18	4-6,10, 11,13	Relevant to claum No.								KKCT N/CK

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Commensus DOCUMENT CONIDERED TO BE RELYANT

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# INTERNATIONAL SEARCH REPORT

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PCT/JP 95/01599	Publication date	31-10-95 05-05-93 31-03-94	20-01-94 18-01-94 03-06-94									
	Patent family member(s)	5462856 0539518 6502757	4203493 2100616 6153954			-						
		US-A- EP-A- JP-T-	AU-B- CA-A- UP-A-									
	Publication date	06-02-92	19-01-94								-	
	Patent document cited in search report	W0-A-9201810	EP-A-0578962								·	
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